WINTER-RUN CHINOOK SALMON CAPTIVE BROODSTOCK PROGRAM: PROGRESS REPORT THROUGH APRIL 1996

Kristen D. Arkush, Michael A. Banks, Dennis Hedgecock, Paul A. Siri
University of California, Davis, Bodega Marine Laboratory
Scott Hamelberg
U.S. Fish and Wildlife Service

Technical Report 49

November 1996

Interagency Ecological Program
for the
San Francisco Bay/Delta Estuary

A Cooperative Program of:

California Department of Water Resources
State Water Resources Control Board
U.S. Bureau of Reclamation
U.S. Army Corps of Engineers
California Department of Fish and Game
U.S. Fish and Wildlife Service
U.S. Geological Survey
U.S. Environmental Protection Agency
National Marine Fisheries Service

WINTER-RUN CHINOOK SALMON CAPTIVE BROODSTOCK PROGRAM: PROGRESS REPORT THROUGH APRIL 1996

Kristen D. Arkush, Michael A. Banks, Dennis Hedgecock, Paul A. Siri
University of California, Davis, Bodega Marine Laboratory
Scott Hamelberg
U.S. Fish and Wildlife Service

Technical Report 49

November 1996

Interagency Ecological Program
for the
San Francisco Bay/Delta Estuary

A Cooperative Program of:

California Department of Water Resources
State Water Resources Control Board
U.S. Bureau of Reclamation
U.S. Army Corps of Engineers
California Department of Fish and Game
U.S. Fish and Wildlife Service
U.S. Geological Survey
U.S. Environmental Protection Agency
National Marine Fisheries Service

Single copies of this report may be obtained without charge from:

State of California
Department of Water Resources
P.O. Box 942836
Sacramento, CA 94236-0001

Printed by Department of Water Resources Reprographics

SUMMARY

The captive breeding program arose from shared concerns for the fate of the Sacramento River winter-run chinook salmon by federal and state government agencies, commercial and recreational fishing associations, California water users, the University of California, and the California Academy of Sciences. In late 1991, these parties formed the Winter-Run Chinook Captive Broodstock Committee to investigate the feasibility of rearing winter-run fry to maturity in captivity, so that broodstock would be available should the natural run disappear. By early 1992, the committee, through public meetings and consensus decisions, designed and began the captive breeding program.

Rearing facilities at Bodega Marine Laboratory and Steinhart Aquarium were designed and constructed around the 1991 year class of juveniles, which was delivered to the Bodega facility in September 1992. Presently, the combined rearing facilities of both institutions are holding three year classes simultaneously. Offspring from the spawning of wild-caught broodstock at Coleman National Fish Hatchery can now be smolted on a natural schedule and delivered to the broodstock rearing facilities with minimal mortality. Survival to maturity in the rearing facilities has exceeded expectations, averaging 40% per year-class rather than a projected 20%. Rates of growth and sexual maturation have also improved steadily. Ultrasound imaging is now routinely employed to assess the degree of reproductive maturation of captive broodstock. The program has successfully demonstrated the feasibility of rearing captive chinook salmon to maturity and obtaining gametes for artificial propagation. With further improvements in broodstock nutrition and fish health, we expect this program to produce abundant supplies of gametes of known genetic background for the U.S. Fish and Wildlife Service artificial propagation effort.

The captive broodstock program has necessitated and enabled substantial scientific and technical advances in the husbandry, pathology, and genetics of chinook salmon. Bacterial kidney disease has been effectively managed in the captive broodstock program so that it is no longer a threat to its success. However, substantial mortality of the 1991 and 1992 year classes, led to identification of a previously unknown intracellular parasite, presently termed the rosette agent. Gametes from the program were placed under a quarantine in 1994 to prevent the spread of this presumed pathogen, and disease research was made the program's top priority. Molecular genetic analysis of this organism's DNA revealed its similarity to rosette agents isolated from other cultured chinook and Atlantic salmon and allowed development of a sensitive detection method for the parasite. In the meantime, the rosette agent was detected in late-fall chinook of hatchery origin in late 1994, which resulted in the lifting of a quarantine on gametes and the subsequent transfer, in 1995, of 30,000 eggs from Bodega Marine Laboratory to Coleman National Fish Hatchery.

The captive broodstock program has worked in close collaboration with the U.S. Fish and Wildlife Service to promote the genetic conservation of winter-run chinook salmon. Hedrick *et al* (1995) called attention to the potential for reducing the effective size and genetic diversity of a natural Pacific salmon run by introducing fry artificially propagated from a small number of parents. Their analysis of the effective size of the winter-run stock showed, however, that the artificial propagation program to which the captive broodstock program contributes gametes is not likely to have this negative effect and may, instead, be helping to maintain or possibly increase slightly the genetic diversity of the stock.

Development of microsatellite DNA markers, necessitated by the need to determine parentage and run identity in the captive breeding effort, represents a substantial technical contribution of the program. In addition to their application in the captive broodstock program as reviewed in this report, under a separate project these markers are being developed and used for a mixed-stock analysis of juvenile chinook salmon in the Central Valley and in the Sacramento-San Joaquin Delta, where chinook salmon are taken by the state, federal, and agricultural water diversions. These markers are also being used by salmon biologists in Alaska, Idaho, Washington, Louisiana, British Columbia, Ontario, Nova Scotia, Denmark, and New Zealand. Thus, these markers will have an impact on salmon biology far beyond their uses in the captive broodstock program.

In response to the inevitable growth in the technical and budgetary complexity of the program, the Winter-Run Chinook Captive Broodstock Committee has modified its administrative structure. The frequency of its general meetings has decreased, and detailed discussions and planning are now undertaken by a series of smaller, focused subcommittees. The first of these was the Genetics Subcommittee, followed by the Budget Subcommittee, the Fish Health Subcommittee, and, in late 1995, the Technical Subcommittee. Subcommittee members now communicate regularly via the Internet and electronic mail. The committee continues to be a responsive agent for conservation of winter-run chinook salmon.

The captive broodstock program was initiated as a rapid response to the endangerment of the Sacramento River winter-run chinook. Gametes from captively reared broodstock have contributed to artificial propagation of the winter-run population. Problems remain, particularly with gamete quality, synchronization of spawning, imprinting of fish released into the Sacramento River on Battle Creek (USFWS 1996), and the risk of admixture and hybridization between the winter run and other runs. Nevertheless, the demonstrated ability of the Winter-Run Chinook Captive Broodstock Committee and the program to surmount such problems leads to optimism that the imprinting and admixture problems can also be resolved. More importantly, the scientific and technical advances necessitated by the program represent a legacy to salmon biology far beyond the scope of the program itself.

CONTENTS

Figures

Tables

IMPLEMENTING AGENCIES

Department of Interior

US Fish and Wildlife Service
US Bureau of Reclamation

Department of Commerce

National Marine Fisheries Service

State of California

Department of Water Resources
Department of Fish and Game

Non-Governmental Agencies

University of California, Davis, Bodega Marine Laboratory
California Academy of Sciences, Steinhart Aquarium
National Fish and Wildlife Foundation
Bay- Delta Agreement Category III Program

Non-Profit Organizations

Pacific Coast Federation of Fisherman's Associations
Golden Gate Troller's Association
Tyee Club and Foundation

PARTICIPATING COMMITTEES

Technical Committee

Kristen Arkush
Mike Banks
Randy Brown
Scott Hamelberg
Ken Lentz
Chris Mobley
Tom Nelson
Katie Perry
Nat Bingham*
Dennis Hedgecock*
Paul Siri*

Budget Committee

Stan Barnes
Nat Bingham
Randy Brown
Scott Hamelberg
Dennis Hedgecock
Ken Lentz
John McCosker
Katie Perry
Paul Siri

Genetics Committee

Mike Banks
Scott Hamelberg
Dennis Hedgecock
Phil Hedrick

Fish Health Advisory Committee

Kristen Arkush
Bill Cox
Scott Foott
Ron Hedrick

*Non-voting members.

Chapter 1
INTRODUCTION

Significant reductions in the size and number of runs of Pacific salmon have been well documented and continue to receive widespread recognition in the scientific, popular, and political arenas (Nehlsen *et al* 1991; Nehlsen 1994; Moyle 1993, 1994; Lichatowich et al 1995). As recently as 1969, there were in excess of 100,000 winter-run chinook salmon (*Oncorhynchus tshawytscha*) adults returning to the upper Sacramento River (Fisher 1994). However, a combination of problems has caused their numbers to drop precipitously. Factors such as streamflow and water temperature may have affected the winter run to a greater extent than the other runs. The Department of Fish and Game estimated that 191 winter-run chinook salmon returned in 1991 and that 189 returned in 1994. Since 1994, the runs rebounded somewhat with estimated spawning escapement of 1,361 in 1995 and 1,296 in 1996. Demographic description of the winter-run (Williams and Williams 1991) and description of a life history unique from all the races of chinook salmon (Healy 1991, 1994) provide evidence that this unusual fish is representative of the increasing genotypic and phenotypic variation associated with the lower latitudes of the range of Pacific salmon (NMFS 1990).

Sacramento River winter-run chinook have been distinguished from the other three runs of Central Valley chinook by the phenotypic convention of run timing. Adult migration of returning spawners begins with fish leaving the ocean and migrating through the Sacramento-San Joaquin Delta from November to June, with a spawning season of mid-April to mid-August (USFWS 1993). The National Marine Fisheries Service listed winter-run chinook salmon as threatened under the Endangered Species Act in August 1989 and proposed reclassification as endangered in 1992; winter-run chinook was formally listed as endangered in 1994. Winter-run chinook was listed as endangered under the California Endangered Species Act in 1989.

A multi-agency winter-run chinook cooperative restoration plan was signed in May 1988 with a provision to initiate hatchery propagation at the Fish and Wildlife Service's Coleman National Fish Hatchery on Battle Creek (USFWS 1989). In July 1990, the Fish and Wildlife Service applied to the National Marine Fisheries Service for an Endangered Species Act scientific permit, which included a winter-run captive propagation program (USFWS 1993). Additional studies on egg temperature tolerance, entrainment of fish by screens, and run differentiation were proposed. During initial planning for the Coleman hatchery artificial propagation project, several challenges became clear. New facilities had to be planned and constructed, and new and untested protocols had to be developed on a non-domesticated fish whose life history is unlike other Pacific salmon. One of the difficulties of attempting to artificially spawn winter-run chinook is the protracted spawning period. Arriving at its natural spawning site in an oceanic appearance, winter-run salmon typically become reproductive during the next 4 to 6 weeks. Thus, when trapped in the river for artificial propagation, it is not always possible to determine sex. Early attempts at holding adult winter-run were complicated by high water temperatures, infections of freshwater fungus *Saprolegnia* spp., and difficulties associated with males and females maturing in synchrony.

In the summer of 1990, the president of the Pacific Coast Federation of Fisherman's Association became concerned that the other runs from the Central Valley, which are the basis of the central California commercial ocean fishery, might be similarly impacted. Meetings ensued with the

Regional Director of the National Marine Fisheries Service with a proposal to amend the Coleman propagation program to allow a portion of the hatchery-produced fish to be used in a captive breeding program. Only two other salmon captive breeding programs of significance were in existence at that time. One involved rearing White River chinook in ocean pens in Washington, and the other involved holding Red Fish Lake sockeye in Idaho and at the NMFS Montlake Laboratory.

The National Marine Fisheries Service provided tentative approval to determine if all the agencies could accomplish the formidable task of managing a captive broodstock program. In October 1991, an *ad hoc* "Winter-Run Chinook Captive Breeding Committee" was formed to determine program feasibility (Bingham and Barnes 1992). Biologists from the Department of Fish and Game, Department of Water Resources, National Marine Fisheries Service, and U.S. Fish and Wildlife Service, together with representatives from the commercial and sport fishing industry and the California Water Commission were joined by University of California marine advisors of the cooperative extension. The committee determined that a captive breeding program was justified.

The committee formed a subcommittee to identify potential sites with the facilities and staff expertise to successfully rear and/or hold salmon. Assisted by biologists from the Montlake Laboratory, which was participating in the Red Fish Lake sockeye salmon captive breeding program, the committee selected the University of California, Davis, Bodega Marine Laboratory, and the California Academy of Sciences, Steinhart Aquarium.

The Winter-Run Chinook Captive Breeding Committee, joined by Bodega Marine Laboratory and Steinhart Aquarium biologists, developed preliminary plans and budgets for a captive broodstock program. The committee established that the captive broodstock annual goal would be to receive about 1,000 juveniles from the Coleman National Fish Hatchery propagation program for the captive breeding program. This number was based on constraints due to budgets and available facilities. The committee assumed that starting with 1,000 juveniles would provide an acceptable quantity of fish for genetic diversity if 20% of the juveniles survived to sexual maturation.

The committee agreed that since Bodega Marine Laboratory had more space and a greater capacity to pump a larger volume of sea water than Steinhart Aquarium, Bodega would be the primary site for holding the bulk of the fish and Steinhart would serve as a backup source of mature fish (and gametes) in the event of natural or mechanical catastrophic loss at Bodega. Bodega Marine Laboratory also had more options to introduce salmon into sea water, which requires discharging a large volume of mixed salinity water over a 3-week period, an option not available at Steinhart. Additionally, being a research facility, Bodega Marine Laboratory was better suited to provide the basic and applied research necessary to support the project.

The initial plan specified that about 1,000 pre-smolt salmon would be transported in fresh water to Bodega Marine Laboratory from Coleman National Fish Hatchery. The fish would be moved into sea water (smoltification) at Bodega, and then about 250 smolts would be relocated to Steinhart Aquarium. The initial plan was to maintain about half of each cohort in fresh water at Bodega to reduce the likelihood of disease. After the first 18 months, it became clear that each cohort would have to be reared entirely in sea water to avoid infection with the freshwater fungus (*Saprolegnia* spp.).

It also became evident during this early stage of the project that the potential impact of the captive breeding program on the remaining wild run needed to be identified and evaluated. The National Marine Fisheries Service authorizes the propagation activity under the Endangered Species Act. As such, that agency closely examined the captive breeding proposal for its potential to become a "genetic sink", in which wild

stocks and the genetic resources they represent would be removed from the habitat for captive breeding and lost due to high mortality in captivity. Conversely, if captive breeding success were high and a large number of animals representing low genotypic variability were released to the wild, then serious deleterious effects could result by an actual depression of the total genetic resource. Results of such practices pose serious risks to natural stocks. Therefore, the committee asked Dr. Dennis Hedgecock, a population geneticist at Bodega Marine Laboratory, to summarize these issues and provide guidelines for the program.

Construction of captive broodstock facilities began, pending funding from drought relief funds, the salmon stamp administered by the Department of Fish and Game, and significant support from the Department of Water Resources and the Bureau of Reclamation. Later in the first year, Fish and Game also contributed money from the California Environmental License Plate Fund. A grant to National Marine Fisheries Service provided for multi-year funding. The total annual budget for all components of the captive breeding program averages $1.2 million. Estimated duration of the program was 10 years (1991-92 to 2001-02), which the committee hoped would yield at least six or seven cohorts.

Chapter 2

PROGRAM DESCRIPTION

The goal of the captive broodstock program is to prevent extinction and loss of genetic material. To accomplish this goal we will attempt to rear winter-run chinook salmon under controlled conditions until they become reproductively mature adults. Mature salmon will then be used as hatchery broodstock for continued propagation of the race. The captive program provides:

- A source of gametes for the Coleman National Fish Hatchery winter-run propagation program.
- A source to supplement naturally spawning salmon.
- A means to "buy time" until habitat conditions in the Sacramento River improve.
- An egg and fry source for experimental studies.
- A maximization of future options for the recovery of the species.

Propagation Program at Coleman National Fish Hatchery

The Fish and Wildlife Service committed to developing a hatchery propagation program at Coleman National Fish Hatchery to assist restoration of winter-run chinook salmon, *Oncorhynchus tshawytscha*. The objective is to capture adult winter-run chinook salmon in the Sacramento River and increase survival of the resultant eggs and juveniles in the protected hatchery environment. This propagation program was designed to supplement the wild run and not to develop an adult return to the hatchery.

Winter-run chinook salmon adults for this program are captured at Keswick Dam fish trap and Red Bluff Diversion Dam fish trap in the upper Sacramento River. The Keswick Dam fish trap is operated from mid-December through July; trapping at Red Bluff Diversion Dam usually begins in mid-May and continues into July. A maximum of 15% of the estimated run can be collected for the propagation program. From the trapping site, adults are transported in water containing artificial slime and/or salt to reduce stress.

When adult fish arrive at Coleman, a numbered Petersen disc tag is attached to each individual to assign an identity. This includes adults captured at any of the trapping sites and adults transported from the captive broodstock program at Bodega Marine Laboratory or Steinhart Aquarium. The tag is affixed with a single wire below and slightly behind the dorsal fin and remains in place until the fish spawns or dies.

Upon arrival at Coleman National Fish Hatchery, adults are placed in the secluded, temperature-controlled broodstock holding tank. The tank is a 20-foot-diameter circular fiberglass unit with a volume of about 5,900 gallons. Water inflow to the tank is normally 100 gpm but is adjusted as necessary. The holding tank is enclosed by a canvas curtain and is equipped with three full-spectrum incandescent lights. Photoperiod and light intensity are controlled by automatic timers and rheostats. Lights are set to go on and off at separate 20-minute intervals to simulate gradual light changes of sunrise and sunset. General photoperiod operation consists of increasing light exposure 0.5 to 1 hour per week beginning in

March. A target date for maximum exposure (15-16 hours) is set for late-April/early-May. Maximum exposure is then maintained until spawning is complete. Water temperature is monitored with thermographs and maintained in the low-50-degree Fahrenheit range with the use of chillers.

Prophylactic and therapeutic treatments (chemical baths and injections) are administered as necessary to assure maximum survival to maturation. Soon after capture, injections of oxytetracycline (20mg/kg fish, intraperitoneal) and erythromycin (20 mg/kg, intramuscular) may be given as a prophylactic treatment for bacterial infections. Additional erythromycin treatments may be given to reduce the potential vertical transmission of *Renibacterium salmoninarum*. If bacterial infection is a suspected cause of morbidity, additional oxytetracycline injections will be given. External fungal infection is controlled by treating adults with a 1-hour static bath of 1-ppm malachite green 3-5 days per week. A single or series of intraperitoneal injections (30 µg luteinizing hormone-releasing hormone analog {LH-RH_a}/kg fish) or time release implants (30 µg LH-RHa/kg fish) are administered to accelerate maturation if death appears imminent or to attempt synchronized maturation (maximum allowable dose is 100 µg/kg). At the time a fish spawns or dies, samples of tissues or fluids may be collected for standard virological and bacteriological assays and histological examination. Scales are also collected for aging.

The Technical Committee discusses mating protocols before each year's spawning season to attempt to fully use all genetic material and minimize or eliminate inbreeding. After 1996, genetic analysis will also be conducted to ensure hybridization is not occurring between chinook salmon runs. Actual design and execution of all matings is conducted by the Fish and Wildlife Service, with input from the Genetics Management Subcommittee. All matings of wild adults, captured returning hatchery fish, or captive broodstock entail efforts to mate unrelated individuals. Relatedness of individuals may be determined through tag recovery (PIT[1] or CWT[2]) or genetic analysis, and no half- or full-sibling matings are expected.

The Fish and Wildlife Service plans to use only wild-caught adults in winter-run spawnings and does not intend to be using progeny from past captive spawnings for these purposes. This practice limits captive rearing to one generation, thus avoiding (as far as possible) domestication and continued enhancement of the same genotypes. Failure to capture sufficient numbers of males and females and adequate genetic variation from the wild, however, may necessitate limited and genetically controlled incorporation of captive progeny into matings. We hope success in capturing wild-caught winter-run spawners will continue to improve, but the captive broodstock provides the only resource available should the wild stock suffer catastrophic decreases.

At the hatchery, winter-run chinook spawn during May, June, and July, consistent with observations of spawning in the wild. To minimize genetic impacts of the winter chinook salmon propagation program on remaining wild stocks, several additional precautions are taken. Eggs from each female are divided into two lots and, when possible, each lot is fertilized with sperm from two males. Also, hatchery staff tries to use each male at least twice, with two females. The practice of creating family groups increases genetic diversity and safeguards against the loss of genetic contribution from an individual producing viable gametes mated with an individual producing nonviable gametes.

Eggs from individual matings are incubated separately in upright incubator trays. To

1 Passive integrated transponder.
2 Coded wire tag.

reduce the potential for vertical transmission of pathogens from the adults, eggs are water-hardened in an iodophor solution (75 ppm active iodine) before they are placed in the incubator (Elliot and Amend 1978; USFWS Fish Health Policy 1995). Incubation water may also pass through an ozone treatment process before reaching the eggs to reduce potential for horizontal transmission of pathogens via the ambient water supply. Incubating eggs also receive a daily, 1-hour flow-through treatment of 167-ppm formalin to reduce losses associated with fungal growth.

Incubation water temperature is held in the low-50-degree Fahrenheit range. Eggs hatch in about 50 days, and alevins are removed from the incubation units after a total incubation period of 2 to 2.5 months. Upon removal, juveniles from individual family groups are moved to one of twenty small (30-inch diameter, 10.2 cubic feet) circular tanks. Since they were installed in 1992, these tanks have proved successful in starting small lots of winter chinook salmon. When all 20 round tanks are full and additional circular tanks are needed, the largest juveniles are moved to one of 49 rectangular (96 cubic feet) tanks. Water flow in all tanks is maintained and adjusted as necessary to provide adequate water quality and maximize growth. Water may also be passed through an ozone treatment system. In the event of water loss after normal working hours, an alarm system will alert on-site resident staff.

Juveniles are started and reared on commercially available diets. The manufacturer's recommended feeding rates are followed as general guidelines but may be altered based on observations of feeding activity. Automatic 12-hour belt feeders eliminate the need for hand feeding, thus potentially reducing adverse behavioral modification. Uneaten food, fish waste, and other debris is removed from rearing tanks daily. To reduce the risk of disease transmission, nets, brushes, and other equipment are thoroughly disinfected between use. Chemical baths and/or oral doses are administered as necessary to maximize survival. Facility construction and modification and development of rearing techniques will continue at Coleman National Fish Hatchery to maximize the health and survival of juvenile winter chinook salmon.

Attempts are made to rear all family groups separately prior to differential marking. If consolidation of family groups becomes necessary, it is done on the basis of maternal half-siblings when possible. All remaining family groups are coded-wire tagged with a unique tag code before release into the river or transfer to off-site rearing locations. The necessity of tagging all families individually is being discussed.

The Fish and Wildlife Service confers with the Genetics Management Subcommittee, National Marine Fisheries Service, Department of Fish and Game, and others to discuss potential release strategies and determine the most effective use of the juveniles. The Fish and Wildlife Service continues to evaluate the potential effect of each release on the effective population size (N_e), as described in its 1993 Biological Assessment (USFWS 1993) and formalized by Hedrick *et al* (1995). As a result of creating many family groups to increase genetic diversity of the hatchery population, the overall impact of the hatchery releases in 1991-1995 on N_e has largely been positive.

Current release strategies of Coleman National Fish Hatchery winter-run chinook salmon juveniles are designed to maximize returns to the upper river. Juveniles are released near Redding (river mile 298) before smoltification to encourage imprinting on the upper river. In the event of poor in-river conditions, designated alternate release sites are Bonnyview Boat Ramp (RM 292), Anderson River Park (RM 283), or North Street Bridge (RM 284). These release sites were selected to avoid developing a hatchery run to Battle Creek and promote homing to and subsequent spawning in an area between river miles 276 and 302. In this reach, suitable water temperature for fry can be maintained with cool water from Keswick and Shasta dams. Releases of

juvenile winter chinook have normally been in mid- to late-January, which falls within the winter chinook salmon outmigration period of about October through March. The fish are released at dusk to assist with acclimation and reduce harassment by predators.

Fish and Wildlife Service surveys in 1995 indicate the release strategy described above may not be sufficient to induce imprinting to the mainstem Sacramento River. The surveys concluded that most hatchery-origin winter chinook salmon adults escaping to the upper Sacramento River in 1995 returned to Battle Creek and not to the mainstem Sacramento River (USFWS 1996). Beginning with broodyear 1995, the Fish and Wildlife Service released the juveniles in December to assist with the imprint. To overcome the problem with imprinting, alternative rearing/release strategies are being proposed.

As shown in Table 1, up to 1,000 juveniles per brood year have been withheld from the general release group for use by the captive broodstock program. These juveniles are marked with PIT tags to allow future identification of an individual to a family group. After PIT-tagging, the juveniles may be pooled into a single group and smolted at Coleman National Fish Hatchery or Bodega Marine Laboratory. Four weeks before their expected introduction to salt water, juveniles are vaccinated against saltwater *Vibrio* spp. Another vaccination follows about 6 months later.

Table 1
EGGS COLLECTED, JUVENILES RELEASED, JUVENILES TRANSFERRED, AND EGG-TO-RELEASE SURVIVAL RATES AT COLEMAN NATIONAL FISH HATCHERY, BROODYEARS 1991 THROUGH 1995

Brood Year	Eggs Collected	Juveniles Released	Juveniles Held for Captive Broodstock Program	Percent Survival[2] (egg to release)
91	29,475	11,582	1,000	43
92	59,445	28,099	1,000	49
93	47,175	18,723	1,000	42
94	61,814	43,346	675	71
95	83,005	51,267[1]	670	61
Average				53

1 BY95 release data include 1,131 juveniles from captive broodstock matings.
2 Includes juveniles assigned to broodstock program.

Bodega Marine Laboratory

All pre-smolts received by Bodega Marine Laboratory are divided into two groups for seawater acclimation. Smoltification transpires over a 17-day period (range 15-21 days) by gradual introduction of sea water to a final salinity range of 32-36 ppt. Salt water addition begins when all the fish have recovered from translocation and demonstrated active feeding for at least 2 weeks. Fish smolted at Coleman National Fish Hatchery are transported in artificial sea water. Once acclimated to the temperature of the BML holding tanks, they are moved into the seawater tanks and distributed to achieve a biomass not to exceed 8.0 kg/m^3.

There are two enclosures at Bodega Marine Laboratory, Sheds 1 and 2. Shed 1 contains eight circular tanks (12 feet diameter, 6 feet deep), which hold about 4,650 gallons of water at operating depth, and two smaller circular tanks (6 feet diameter, 4 feet deep). Each tank has a central drain and provides circular water movement by a surface inlet at a maximum flow rate of 50 gallons/minute. Flow rate is adjusted to maximize growth. Each of the 12-foot-diameter tanks has an observation window to examine feeding behavior and tank condition. Shed 2 contains nine circular tanks (4 feet diameter, 4 feet deep) and six larger circular tanks (6 feet diameter, 6 feet deep).

Both sheds provide flow-through sea water and fresh water. All fish are sustained with water filtered to eliminate all particles 20 microns and larger and UV sterilized at a minimum of 30,000 mws/cm^2. Filters are sand (prefilter) and pleated cartridges. The entire facility, including pumping equipment, is covered and enclosed to minimize incidental contamination by humans and animals. Windows have been installed to allow adequate ambient photoperiod. To minimize losses due to mechanical failure, pressure, temperature, and low-water alarms have been added so that staff can be called to the site at any time.

Bodega Marine Laboratory has the capability of generating ozone for disinfecting sea water and fresh water. Plans to ozonate effluent water are underway, and experiments are underway to test the feasibility of disinfecting influent water by ozonation. If successful, ozone may be used in addition to, or in lieu of, ultraviolet disinfection for incoming water.

Diet

Initially, the juveniles are fed a standard moist pellet formulation (BioDiet) by hand to closely monitor consumption rates. After the fish are acclimated to the Bodega Marine Laboratory environment, the pelleted diet is delivered by automatic feeders at 2-4% body weight. The pellet formulation is then reduced, and the fish receive a diet at 2-4% body weight consisting of 80% krill (*Euphasia pacifica* and *E. superba*) and 20% cut anchovies, with some pellet supplementation. Feeding is spread throughout the day. Tanks are monitored closely to ensure that the fish are not overfed and that any uneaten food is siphoned away every 6-12 hours (or sooner).

Bacterial Standards

The freshwater and seawater systems are both assayed bimonthly for bacteria upstream from the mechanical filters and upstream from the ultraviolet source to determine efficacy of the UV and filtration units. All mechanical filters are monitored daily and cleaned weekly. As needed, filter cartridges are rotated with chlorine-sterilized cartridges. Ultraviolet bulbs are dated and rotated so that dosage deterioration is limited to 50% of the expected life on half of the bulbs. All bulbs are housed in quartz sleeves for maximum effective kill. Moribund and diseased fish are removed promptly and kept in the containment facility for analysis. Nets, buckets, and other tank-related equipment are dedicated to specific tanks and sterilized after each use. Nets and other incidental equipment are not introduced to the facility from other areas of the laboratory.

Emergency Plan

Temperature changes, loss of water pressure, and low water levels have automatic alarms. During non-business hours the alarms are included in regular callback procedures of Bodega Marine Laboratory. All project personnel are on an established callback roster. Loss of electrical service (Pacific Gas and Electric Company) automatically switches the facility to Bodega Marine Laboratory's backup generator (500 kW). Should the backup generator fail, a secondary backup generator (175 kW) comes on line. The system design includes two small (5 HP) chillers to work in tandem with the centralized chillers. This design allows one system to go down without affecting temperature control.

Bodega Marine Laboratory has an automatic security gate operating during non-business hours to help minimize vandalism. Personnel are present every day to provide surveillance.

Steinhart Aquarium

At Steinhart Aquarium, all fish are held in either of the two systems dedicated to winter-run salmon and separate from all other aquarium life-support systems. One system is tank 11 of the public aquarium, a 6,495-gallon seawater tank (about 17.5 feet x 9.5 inches x 6 feet) with rounded lateral edges. One face of the tank has two 5x5-foot viewing windows; the rest of the tank is coated with a smooth blue epoxy finish. Water current is clockwise and is generated by a 50-gpm circulation pump. Sea water is sequentially filtered down to 10 microns by passive flow-through filter floss, followed by pressurized flow through a pleated cartridge filter. The sea water is pumped from the ocean at the south end of the Golden Gate Bridge. The sea water is refrigerated by redundant 5-ton chillers and recirculated by redundant 50-gpm pumps. It is UV-sterilized at a minimum of 30,000 mws/cm^2. Lighting is synchronized with the natural photoperiod using a time clock.

The second system is isolated from tank 11 and is in an enclosed garage with a high ceiling. It consists of two cylindrical fiberglass tanks (12 feet diameter, 6 feet deep) with slightly conical bottoms to allow self-cleaning. Each tank has a central drain, and circular water movement is provided by a surface inlet at a maximum flow rate of 100 gallons/minute. The life-support system for the second system is similar to that of tank 11 but is larger in size and capacity. Lighting is synchronized with the natural photoperiod using an automated sidereal calendar control. All tanks are covered with mesh netting to prevent escapement.

Diet

The diet consists of moist salmon pellets, *E. pacifica*, *E. superba*, and cut anchovies. Fish are fed throughout the day (about 3 times/day), and uneaten food and accumulated waste is removed daily.

Bacterial Standards

A bacteriological assay is done bimonthly on each recirculation system upstream and downstream of the ultraviolet unit to determine water quality and percent bacteriological kill. Maintenance logs are kept so that UV bulbs are changed at least every 6 months so as not to exceed 10 colony-forming units (cfu) per milliliter of sampled water. Moribund and diseased fish are removed promptly for analysis. Equipment is not shared between tanks, and systems are dismantled and chlorine disinfected between use.

Emergency Plan

Additional tanks of similar volume and dimensions are available on an emergency basis. A transport vehicle and tanks are available if the fish must be moved to another site. A 65-kW emergency generator with an automatic transfer switch is dedicated to the winter-run life-support system supplying the fiberglass tanks in the garage. Electrical connections also exist between both winter-run systems and the aquarium's two 65-kW emergency generators in case of failure of the primary emergency generator. Temperature, flow rate, and water levels of both systems have automatic alarms. Redundant equipment is used to protect against equipment failure. Staff is on-site and available at all times.

Egg Transportation

Upon reaching sexual maturity, fish held at Steinhart Aquarium or Bodega Marine Laboratory may be spawned and their gametes prepared for shipment to Coleman National Fish Hatchery as fresh milt and unfertilized or "green" eggs. Once the eggs are collected, they are placed in plastic zip-lock bags, with eggs not to exceed three layers in the bag. The bags are filled with pure oxygen, wrapped in wet cheesecloth, and loaded into Styrofoam egg-shipping cartons, one bag per tray. Crushed ice is placed on the top tray, and the carton is sealed and transported directly to Coleman National Fish Hatchery for fertilization with milt from a wild-captured winter-run male. Milt is packaged similarly and shipped to the hatchery to fertilize eggs from wild winter-run females.

In the absence of wild winter-run males to fertilize eggs from the captive broodstock, milt may be collected from jacks of other broodyear classes of captive winter-run fish (fresh or cryopreserved). This milt is combined with the eggs at the captive broodstock site, and the fertilized eggs are disinfected with povidone-iodine at 100 ppm for 15 minutes, then water-hardened for 30 minutes. Fertilized eggs are packaged and shipped as described for gametes, except that they are not placed in oxygenated, zip-lock bags.

Egg Incubation at Bodega Marine Laboratory

To accommodate on-site rearing of captive winter-run chinook eggs at Bodega Marine Laboratory in 1995, a small recirculation hatchery system was installed in the northeast corner of Salmon Shed 1. The system consists of a holding sump (18" x 19" x 89"), one upwelling jar (12-3/4" x 4"), and four standard Heath trays (15" x 21-1/4" x 2"). As a disease consideration, the trays and upwelling jar are arranged on a water table so that the water is delivered to each unit separately. After passing through the jar and trays, the system water returns to the sump, where it mixes with the make-up water at 1 gpm. Water pumped from the sump is processed through a 1-ton chiller, a sand filter canister filled with clinoptilite, and a 20-micron Jacuzzi pleated canister and is then UV-sterilized at a minimum of 30,000 mws/cm2. Each tray receives water flow of 4 gpm, and the upwelling jar receives 0.75-1 gpm to maintain a gentle rolling action of the eggs.

Make-up well water is supplied to the system at a rate of 1 gpm, and the temperature is maintained at 10-12°C through the use of a temperature controller and an additional side-stream chiller loop. The system may be shut down periodically for up to 20 minutes at a time for cleaning, filter changes, and clinoptilite rejuvenation. All eggs receive a standard formalin treatment three times per week at 1667-ppm flow-through for 15 minutes. Formalin treatments are discontinued at first hatch.

At swim-up, the 4 trays and the upwelling jar are removed and replaced with five large, acrylic aquaria (24" x 13" x 19-3/4"). The fish are transferred to the aquaria, and water flows are increased to 6 gpm with aeration. Automatic belt feeders positioned over each aquarium deliver feed throughout the day and also act as lids to prevent escapement. When feeding commences, an additional filter bag constructed of nitex screen is added to the system to trap uneaten food and feces. Aquaria are siphoned and mortalities are removed daily.

Chapter 3
RESULTS

The first year class to be reared at Bodega Marine Laboratory, broodyear 1991 (BY91), arrived September 16, 1992, at 14 months of age. Both BY92 and BY93 arrived at 7 months of age. Relative growth of each of these year classes, by weight, is shown in Figure 1. The graph shows growth data collected from the time of PIT tagging only. BY93 fish grew appreciably as compared to those from BY91 and BY92. Two significant factors could have caused this difference.

- BY91 and BY92 suffered multiple outbreaks of bacterial kidney disease (BKD) and were treated with erythromycin by feed. The fish rejected medicated feed and fed less aggressively once returned to their unmedicated diet.
- Delayed PIT tagging of the BY92 salmon delayed their introduction to sea water, which caused poor smoltification. Introduction to sea water was also delayed for the BY91 (groups were moved to sea water at 19-22 months). For BY91, seawater phasing occurred within the observed period of winter-run chinook outmigration, yet these chinook had undergone smoltification as age-1+ fish. BY92 phasing was attempted at 13 months of age, at a time of year outside the observed window of natural smoltification.

Figure 2 shows growth in fish held at Steinhart Aquarium. Difference in size between BY93 cohorts reared at Bodega Marine Laboratory and those reared at Steinhart Aquarium is partly due to limitations of the recirculation system at the aquarium. Biological loading and feeding rates are limited by ammonia production and the capacity of the filtration system to remove nitrogenous waste. For comparison, Figure 3 provides weight measurements for BY93 fish at Bodega Marine Laboratory and at Steinhart Aquarium.

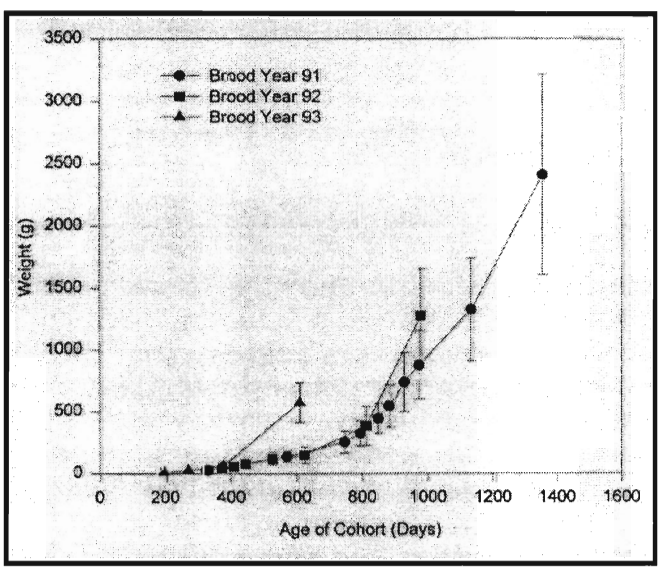

Figure 1
WINTER-RUN CHINOOK SALMON CAPTIVE BROODSTOCK COMPARATIVE GROWTH, BY COHORT, BODEGA MARINE LABORATORY

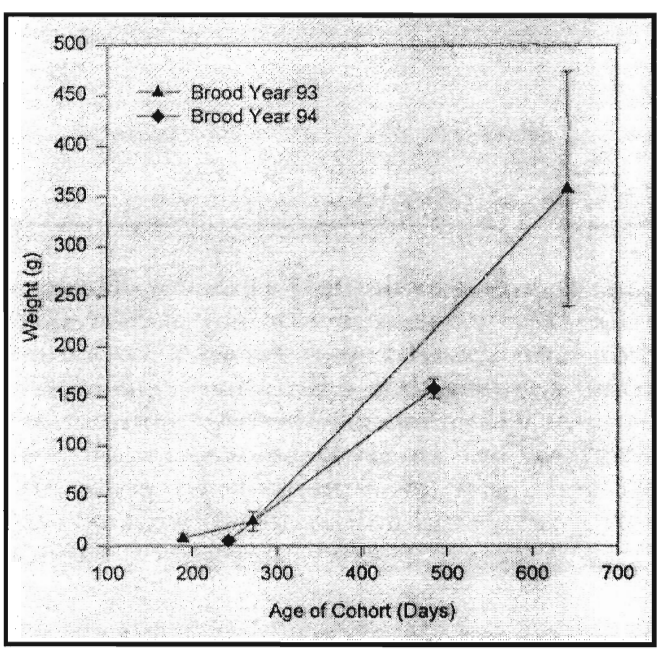

Figure 2
WINTER-RUN CHINOOK SALMON CAPTIVE BROODSTOCK COMPARATIVE GROWTH, BY COHORT, STEINHART AQUARIUM

Ultrasound

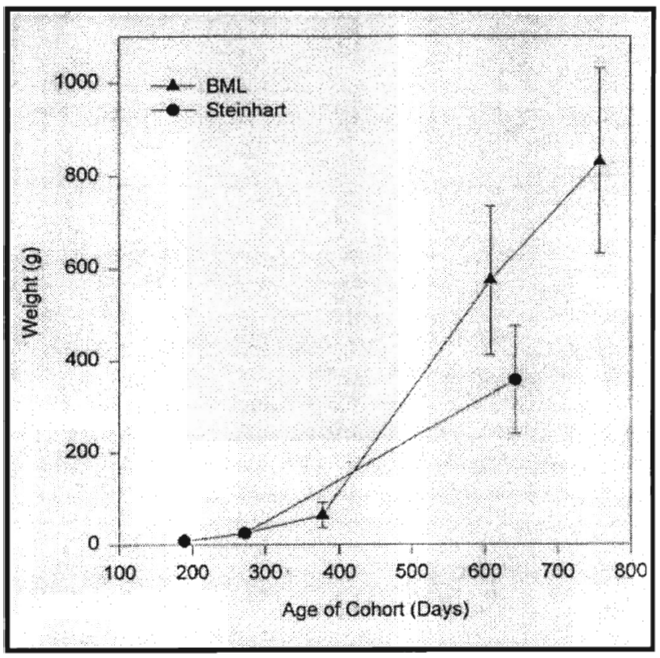

Figure 3
WEIGHT COMPARISON FOR BROODYEAR 93

Beginning with the 1995 spawning season, reproductive maturation in the captive broodstock has been assessed using ultrasound imaging in addition to traditional husbandry techniques such as palpation. Separation of maturing fish from reproductively immature animals by ultrasound allows for segregation of spawning candidates and reduced handling of immature fish. This technique was first described for use in determining salmonid sex and maturation in Norway (Reimers *et al* 1986) and is now part of routine aquaculture practice in that country. The Winter-Run Chinook Captive Broodstock Program is the first project of its type in the United States to incorporate ultrasound imaging in husbandry practice. Anesthetized fish are scanned using a dual frequency (5.0 and 7.5 MHz) linear probe. Images can be saved digitally and imported into the computerized database. In this way, gonadal development and maturation of a particular fish can be followed over time. This information, along with genotypic analysis, can identify broodstock that might contribute to the winter-run chinook propagation program.

Ozone Analysis

Experiments to evaluate the efficacy of ozone to disinfect sea water for captive broodstock life support began in 1995. Ozone is produced using an OREC model SP3-AR ozonator with a model DM-100 monitor. An initial design to treat seawater effluent involved batch treatment of water in an in-ground 1,500-gallon tank, with ozone bubbled into the water to achieve a final hydraulic residence time of 18 minutes. Measurements of total residual oxidants by the indigo method (Greenberg *et al* 1992) and bactericidal activity of the oxidants by bacterial plate count (Greenberg *et al* 1992) showed that this initial treatment regime was ineffective. A newly installed Mazzei venturi inline injection system is being evaluated, with promising results.

Maturation in the Captive Broodstock

To date, multiple year classes of captive winter-run chinook salmon have demonstrated early male maturation as either year-1 (precocious) or year-2 (jack) fish. This asynchronous maturation and early death reduces the effective genetic contribution of males during the spawning season. Only some of these males produce milt acceptable for cryopreservation, and this frozen milt is typically inferior (in terms of fertilization success) to fresh milt. With respect to the BY91 cohort, the population surviving as age-4 fish was almost exclusively female. Early male maturation is evaluated at necropsy, either with the release of milt or by the presence of well-developed gonads in the absence of actual spermiation. Early-maturing males are more likely to die prior to successful reproduction in the captive broodstock program because these fish experience difficulty in osmoregulation during their reproductive phase.

For broodyears 1991 to 1994, incidence of year-1 (precocious) and year-2 (jack) male maturation is shown in Figure 4 for fish held at Bodega Marine Laboratory and in Figure 5 for fish held at Steinhart Aquarium. Fish releasing milt and those with developed testes are both considered as "mature". At Bodega Marine Laboratory, 0.7% and 24.8% of the BY92 males died as year-1 and year-2 fish, respectively (Figure 4). BY93 had fewer losses from early maturation; only 3.0% died as year-1 males and 6.8% died as year-2 males. By comparison, 1.3% of the BY93 males held at Steinhart Aquarium were precocious, and 1.0% developed as jacks (Figure 5). Of the BY94 males, 1.2% died as year-1 males and 7.7% died as year-2 males. Efforts to reduce this early maturation and consequent loss of genetic contribution, such as restricting feeding rates for the males during fall and winter, are now being considered as potential management tools.

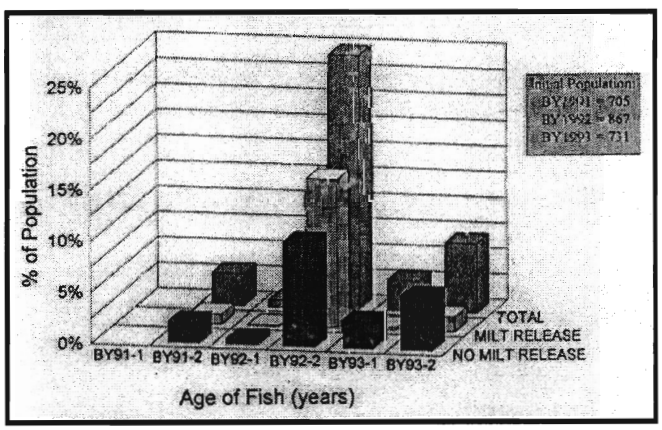

Figure 4
EARLY SEXUAL MATURATION IN CAPTIVE BROODSTOCK MALES REARED AT BODEGA MARINE LABORATORY AS DETERMINED AT THE TIME OF NECROPSY
Percentages of year-1 (precocious) and year-2 (jack) males are calculated from the total initial population (malaes and females combined for a given broodyear). Both firsh releasing milt and those with developed testes are shown.

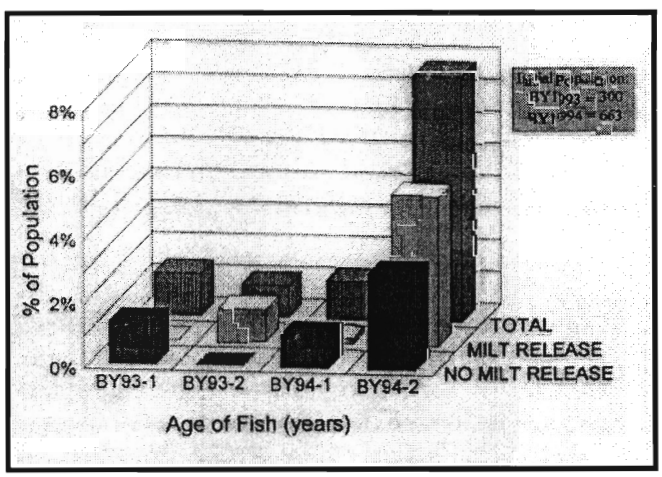

Figure 5
EARLY SEXUAL MATURATION IN CAPTIVE BROODSTOCK MALES REARED AT STEINHART AQUARIUM AS DETERMINED AT THE TIME OF NECROPSY
Percentages of year-1 (precocious) and year-2 (jack) males are calculated from the total initial population (malaes and females combined for a given broodyear). Both firsh releasing milt and those with developed testes are shown.

Spawning Success in 1995

In 1995, the captive broodstock program met a significant program goal by providing gametes to the propagation program at Coleman National Fish Hatchery (Table 2). Eighteen females (14 BY91 and 4 BY92) were spawned by Bodega Marine Laboratory staff, and the unfertilized eggs were shipped to Coleman for fertilization with milt from wild winter-run fish. In addition, three captive-reared females (BY91) were crossed with captive-reared jacks, and these fertilized eggs were transported to Coleman for rearing and potential release. Augmentation of the propagation program, specifically with fish from the BY91, was considered critical this year in light of the population size estimate of only 191 wild winter-run fish for 1991.

In 1995, five additional matings were conducted using BY91 females and BY93 jacks (Table 3), but surviving progeny remain in captivity. These experimental crosses have provided information on the feasibility of using alternative rearing units (Heath tray versus upwelling jar) and fertilization rates of eggs using fresh versus cryopreserved milt. Also, some of these captive-by-captive progeny are being used in an experiment to examine the potential role of vertical transmission in the pathogenesis of the rosette agent.

Traditional hatchery practices rely almost exclusively on Heath trays for egg incubation, despite the risk of fungal contamination and spread. Upwelling jars are less common, yet their design is space efficient and may better mimic in-river conditions as the eggs are gently rolled in the unit. Additionally, dead eggs rise to the surface in these units and are easily removed from the top without disturbing the viable eggs during sensitive early incubation. To compare hatch success in traditional Heath trays with that of upwelling jars, the eggs of one female were divided equally and reared to hatch in both types of units. Of the eggs reared in the tray, only 38% reached eye-up. At 63%, the percent eye-up in the upwelling jars was markedly higher. However, development beyond the eye-up stage (hatch success) in the tray and jar was nearly identical.

To test the relative performance of cryopreserved milt versus fresh milt and to evaluate our cryopreservation methods, eggs from a single female were divided equally and fertilized with either fresh or cryopreserved milt from the same male. In this instance, eggs fertilized with cryopreserved milt had a much poorer hatching rate (5%) compared to eggs fertilized with fresh milt (53%). Reduced fertilization success using cryopreserved material is not uncommon, yet further work to improve methods for cryopreservation may prove beneficial to this and other captive broodstock programs, where conservation of genetic resources is of great importance.

The captive broodstock program and Coleman National Fish Hatchery are participating in an Investigational New Animal Drug field trial (INAD 8061) for the use of luteinizing hormone releasing hormone analog (des-Gly^{10},[D-Ala^{6}]-LH-RH Ethylamide, or $LHRH_a$). Beginning in 1995, spawning candidates in the captive program were injected with $LHRH_a$ in an effort to promote final egg maturation and release (Weil *et al* 1978; Donaldson *et al* 1981, 1985).

Spawning success in captive-reared winter run chinook was lower than that of wild fish spawned at the Coleman National Fish Hatchery in 1995 (Table 2). Wild winter-run chinook spawned at the hatchery had a hatching rate of 74-85%, while crosses using gametes from captive broodstock resulted in 0-66% hatching success. Several factors may have contributed to this disparity. Disease management strategies precluded the movement of spawning adults from the captive broodstock program back to Coleman National Fish Hatchery, so only unfertilized eggs were transported. However, the effects of transporting eggs prior to fertilization are unknown. Spawn

Table 2
SUMMARY OF GAMETES COLLECTED IN 1995 AND RESULTS OF FERTILIZATION

Spawn Date	Case #	Female PIT Tag	CNFH Lot	Destination	Male	% EYE-UP	% HATCH
7/10/95	B91-632	7F7D767726	3A	Green Eggs to CNFH	CNFH - N	85.4	65.6
					CNFH - O	83.8	62.9
7/18/95	B91-636	7F7D7D537C	3A	Green Eggs to CNFH	CNFH - K	23.4	3
					CNFH - I	16.5	1.5
7/28/95	B91-640	7F7D767831	1	Green Eggs to CNFH	CNFH - T	67.5	43.7
					CNFH - R	72.7	55.6
8/1/95	B92-840	006328258	K15	Green Eggs to CNFH	CNFH - R	50	13.2
					CNFH - U	44.1	0.9
	B91-643	7F7D7D1B74	1	Green Eggs to CNFH	CNFH - U	11.7	7
					CNFH - R	8.9	1.8
	B91-645	7F7D01302B	3A	Green Eggs to CNFH	CNFH - T	0.7	0.2
					CNFH - N	0.1	0
	B91-646	7F7D7D213E	3A	Green Eggs to CNFH	CNFH - K	0	0
					CNFH - M	0	0
	B91-647	7F7D7B354C	3A	Green Eggs to CNFH	CNFH - V	0.3	0
					CNFH - P	0	0
	B91-648	7F7D767837	3A	Green Eggs to CNFH	CNFH - M	0	0
					CNFH - W	0	0
	B91-649	7F7D78382B	3B	Green Eggs to CNFH	CNFH - S	14.6	4.2
					CNFH - L	12.7	5.3
8/3/95	B91-650	7F7D766F6C	3A	Green Eggs to CNFH	CNFH - R	0	0
					CNFH - U	0	0
	B92-841	005340826	F11	Green Eggs to CNFH	CNFH - T	0	0
					CNFH - W	0	0
	B92-842	005622270	J15	Green Eggs to CNFH	CNFH - M	4.6	0.6
					CNFH - P	12.8	3.1
	B92-843	005370633	C4	Green Eggs to CNFH	CNFH - S	39.1	31.4
					CNFH - K	49.1	38.9
8/8/95	B91-653	7F7E664058	3B	Green Eggs to CNFH	CNFH - U	0	0
					CNFH - R	0	0
	B91-654	7F7D4A2440	1	Green Eggs to CNFH	CNFH - V	45.2	32.5
					CNFH - W	51.7	35.2
	B91-655	7F7D30044E	1	Green Eggs to CNFH	CNFH - S	6.8	1.4
					CNFH - K	5.9	0.9
8/11/95	B91-657	7F7D783768	1	Green Eggs to CNFH	CNFH - S	38.5	31.4
8/17/95	B91-658	7F7D784572	3B	Fertilized Eggs to CNFH	SA BY93 1F430E6729	0	0
					SA BY93 1F49556063	0	0
					SA BY93 1F4F3B4F08	0	0
	B91-659	7F7D4A1F0C	1	Fertilized Eggs to CNFH	BML BY93 1F43314F1E	0	0
					SA BY93 1F3F761715	0	0
	B91-660	7F7D783525	3A	Fertilized Eggs to CNFH	SA BY93 1F4F304E14	0	0
					SA BY93 1F4969456A	0	0

Table 3
SUMMARY OF CAPTIVE-BY-CAPTIVE CROSSES HELD AT BODEGA MARINE LABORATORY

Spawn Date	Male Broodyear and PIT Tag	Female Broodyear and PIT Tag	Milt Type	Rearing Vessel	Green Eggs	% Eye-Up	% Hatch	Number Survived
8-24-95	93-1F4F304E14	91-7F7D767A04	Fresh	Tumbler	604	63	33	170
8-24-95	93-1F4F304E14	91-7F7D767A04	Fresh	Tray 1	1161	38	31	324
8-24-95	93-1F4F427E52	91-7F7D7A166F	Fresh	Tray 2	2547	95	54	1263
8-31-95	93-1F433A2F35	91-7F7D4C2C6C	Cryopreserved	Tray 3A	1336	16	5	57
8-31-95	93-1F433A2F35	91-7F7D4C2C6C	Fresh	Tray 3B	1356	61	53	672
8-31-95	93-1F43447862	91-7F7D2F3D15	Cryopreserved	Tray 4A	1587	38	11	167
8-31-95	93-1F427F4C54	91-7F7D7D3756	Cryopreserved	Tray 4B	2919	4	0.7	20

timing in the captive broodstock was protracted and not synchronized with the wild fish, despite the use of $LHRH_a$. The timing of a number of compounding factors, including freshwater reentry, cessation of feeding, and hormone therapy, coupled with increased handling, may have affected spawning. Last, the captive broodstock is reared on a combination of artificial and natural food items, which may not satisfy requirements for reproductive growth and gamete viability. Reduced gamete quality in captive-reared fish is common to salmonid broodstock programs throughout the Pacific Northwest. We are continuing to evaluate various factors that contribute to gamete quality (eg, diet) to improve hatching success of progeny from captive-reared fish.

Pathology

All fish reared as part of the captive broodstock at Steinhart Aquarium and Bodega Marine Laboratory are subjected to a comprehensive necropsy at time of death. At necropsy, a fin clip is collected as a backup sample for the Genetic Analysis group. Wet mounts of skin scrapings and gill tissue are routinely examined. Additionally, blood smears and/or plasma samples, tissue imprints, and tissue samples for histological examination are collected. When appropriate, bacteriological and virological samples are also collected. For example, ovarian fluid and tissue samples are collected routinely for virus isolation. All tests are conducted in accordance with methods detailed in *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens* (Thoesen 1994).

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is a significant cause of disease in the captive winter-run chinook salmon broodstock. The program has participated in the INAD permit 4333 for erythromycin, coordinated by individuals at the University of Idaho. Kidney samples from all dead fish are screened for the presence of *R. salmoninarum* by the direct fluorescent antibody test, and the relative level of infection is estimated by the enzyme linked immunosorbent assay. When the level of antigen increases, suggestive of an impending outbreak of BKD in the population, fish may be (and have been) treated with erythromycin delivered by medicated feed.

Effective treatment of BKD has been a significant challenge for the captive broodstock program. The parasite is transmitted both horizontally (fish to fish) and vertically (parent to progeny via the egg) (Mitchum and Sherman 1981; Bullock *et al* 1978; Evelyn *et al* 1986). Almost all stocks of

Pacific salmon are infected with the pathogen (Fryer and Sanders 1981). There has been significant year-to-year variation in the onset and severity of infection among the cohorts in captivity. *R. salmoninarum* is an obligate intracellular parasite, so treatment of the disease is difficult, since available chemotherapeutants have no effect on the intracellular bacteria (Wolf and Dunbar 1959). To control the level of infection, however, erythromycin delivered in the feed has been administered to BY91, BY92, and BY93 fish, with varying results. A significant challenge of applying medication by feed is that sick fish are often inappetant and may not consume a therapeutic level of antibiotic. In addition, the medicated feed is less palatable, so the fish often reject feeding altogether. As a result, brood-years 1991, 1992, and 1993 suffered substantial mortality to BKD. The BY94 fish suffered a BKD epizootic at Coleman National Fish Hatchery prior to their transport to Steinhart Aquarium. For this year class, aggressive, early treatment for the disease was initiated. Instead of medicated feed, however, these fish have been treated with erythromycin by injection. Although this method is more labor intensive and requires handling each fish, it has improved control of BKD outbreaks with reduced mortality.

A systemic protist, the "rosette agent", has contributed to morbidity and mortality of the fish held at both Steinhart Aquarium and Bodega Marine Laboratory. A similar parasite has been detected in other Pacific salmonid populations (Harrell *et al* 1986; Hedrick *et al* 1989). Following detection of the rosette agent in the captive population, the Department of Fish and Game restricted the movement of infected fish and/or their gametes. Although this agent has not been detected in wild winter chinook, in 1995 it was detected in returning late-fall chinook at Coleman Hatchery (S. Foott, personal communication). The Department of Fish and Game has since allowed movement of gametes from the captive broodstock back to Coleman, thereby assuring continued contributions of the captive program to propagation efforts. However, methods to detect the parasite, means to control infection, and relative risk of spreading the disease to other populations continue to be studied.

A sensitive method to detect the parasite has recently been developed at the BML Fish Disease Laboratory in collaboration with the Genetic Analysis group. Using sequence information from another rosette agent isolate (Kerk *et al* 1995), a rosette agent-specific genomic DNA sequence can be amplified by the polymerase chain reaction (PCR). Results demonstrated identical sequence from each of these isolates. The primers are specific for rosette agent DNA only and do not amplify product from host (salmon) DNA or DNA from other fish parasites tested to date (PKX, a myxosporean, and *Loma salmonae*, a microsporidian). These primers will be used to amplify rosette agent DNA from host tissue, thereby providing a sensitive detection method for the parasite that may enable identification of the rosette agent during very early stages of infection.

Experiments are also underway to evaluate the efficacy of a rosette agent vaccine. *In vitro* cultures of the parasite were killed with formalin, then used to vaccinate yearling chinook held in fresh water. Another experiment in progress is designed to determine the relative susceptibility of five salmonid species (*Oncorhynchus mykiss*, *O. kisutch*, *O. tshawytscha*, *Salmo trutta*, and *Salvelinus fontinalis*) to infection by the rosette agent. Both experiments are being conducted at the University of California, Davis, Fish Disease Laboratory containment facility.

Assessment of the risk of disease to other salmonids by the rosette agent, as well as technological development in tools of early detection and treatment for the disease, will assist in recovery efforts for the winter-run chinook through the captive propagation program.

Genetic Analysis and Management

From its inception, the Winter-Run Chinook Captive Broodstock Committee recognized the need to evaluate the impact of the artificial propagation and captive broodstock programs on the genetic integrity of the native winter-run stock. A Genetics Subcommittee was formed to tackle three initial genetics management tasks:

- Design mating protocols for the wild-caught spawners at the Coleman National Fish Hatchery.
- Resolve the full-sib and half-sib families in the 1991 year class that had been combined into four tank lots at Coleman National Fish Hatchery.
- Model the effects of artificial propagation on the effective size of the total winter-run population.

Availability of a DNA test for sex made it possible to sex not only captive broodstock but also wild-caught broodstock, which do not normally show secondary sexual characteristics until after they have been transported from the Sacramento River to Coleman National Fish Hatchery.

In 1995, a need became apparent to discriminate other stocks of chinook salmon that might be inadvertently captured along with winter-run broodstock. Molecular tools developed in an independent project for mixed stock analysis of Central Valley chinook salmon provided valuable insight into this consideration.

Development of Microsatellite Markers as Tools for Genetic Management

The 1991 captive broodstock year class was produced at Coleman National Fish Hatchery by 12 pair-crosses among 15 wild-caught winter-run broodstock (USFWS 1992). These 12 families experienced different levels of mortality, so that by the fry stage the smaller families had to be pooled together with larger ones to maintain normal feeding behavior in the hatchery's large production tanks. This constraint was eased the next year by installing 20 smaller tanks for rearing small numbers of fry. In the meantime, it became necessary to identify the parentage of each fish subsequently taken into the captive broodstock program, because parent-fish had contributed unequally to the 1991 year-class of hatchery-produced winter-run. Milt from one of the nine males, for example, had been used to fertilize 61% of the eggs and one of the six females had contributed 41% of all the eggs used in the hatchery crosses (Hedrick *et al* 1995). Without parentage information, the captive broodstock program would have run the substantial risk of over-utilizing gametes from only two of the original 15 founders or of crossing gametes from full- or half-siblings, resulting in inbreeding.

We resolved to use genetic markers to confirm the parentage of the 1991 year-class of captive broodstock. For this task we required genetic markers that could be ascertained in non-harmful biopsy tissue samples and that had sufficient levels of variation to allow unique discrimination of the 15 parents in progeny. Protein polymorphisms that had been widely used in studies of salmon genetics failed both of these criteria. We turned instead to DNA markers that could be amplified by means of the polymerase chain reaction (PCR) from small fin clips, thus meeting the first criterion. Next, we focused on a recently discovered class of genetic markers, called "microsatellites", that have proven abundant and very polymorphic in most higher organisms, meeting our second criterion.

Microsatellites have core sequences 2-6 nucleotides in length that may be repeated from 10 to 100 times at a particular chromosomal site. Hundreds of thousands of microsatellites are scattered throughout the genomes of higher organisms (Tautz and Renz 1984), which has led to their adoption as landmarks in the Human Genome

Project. The number of repeated units in a particular microsatellite is often highly variable within a population due to a high rate of insertion or deletion mutations (10^{-3} to 10^{-4} gametes per generation (Queller *et al* 1993; Weissenbach *et al* 1992; Hearne *et al* 1992; Edwards *et al* 1991)). This rate is three to five orders of magnitude greater than rates of electrophoretically detectable amino acid substitutions or nucleotide substitutions in mitochondrial or coding DNA. Microsatellites, therefore, are more likely to reflect recent evolutionary events. They have, thus, been applied in a wide variety of population genetic studies, especially at the intraspecific and congeneric taxonomic levels (Tautz 1990; Amos *et al* 1993; Queller *et al* 1993; Hughes and Queller 1993).

Standard methods of lysis in a proteinase K solution followed by phenol:choloroform extraction (per Sambrook *et al* 1989) were used to extract genomic DNA from liver tissue of a few individuals for library construction. This DNA was digested with restriction enzymes and size fractionated using agarose gel electrophoresis. Eluted fragments about 250-550 bp were ligated into pBluescript (SK-, Stratagene) cloning vectors. These were then used to transform competent *E. coli* XL1-blue cells (Stratagene) to form a library containing randomly sampled fragments from the chinook salmon genome.

This library of salmon DNA was screened with a variety of di-, tri-, and tetra-oligonucleotide probes (QUICK-LIGHT, FMC) corresponding to alternate microsatellites found to be common in fish. The associated QUICK-LIGHT hybridization kit allows identification of clones containing chinook microsatellite DNA inserts within 2-3 hours. Southern blotting was used to confirm that positive clones contained microsatellites before the sequence of the cloned DNA was determined by standard dideoxy-chain-terminating sequencing protocols (USBiochemical, sequenase).

Sequence data for clones containing microsatellites were evaluated for their amenability to enzymatic amplification by PCR. OLIGO (National BioSciences NBI) software was used to design optimal PCR-primers. An in-house Pharmacia Gene Assembler II was used to synthesize primers with 5' fluorescent amidites. The Taguchi method (Cobb and Clarkson 1994) was used for rapid optimization of PCR conditions.

Once optimum PCR conditions were established for loci, we characterized them by screening samples from the captive winter-run broodstock and subsamples from each of the other runs to identify the more informative loci. Six microsatellite markers were developed for winter-run analysis in 1993-94; development of 18 new loci is in progress, and primers for an additional 21 loci have been obtained from laboratories in Nova Scotia, Seattle, and Anchorage. The total number of genetic markers available in the near future may be more than 40 loci. Our FMBIO fluorescent imager (Hitachi), which we use to resolve PCR-product fragments following polyacrylamide electrophoresis, has a number of features that greatly increase the efficiency with which we are able to generate data.

Results from controlled breeding for captive broodstock demonstrate that chinook salmon microsatellites exhibit simple Mendelian inheritance as codominant markers (Banks *et al*, in prep). Inference for populations under consideration thus involves simple and direct application of general population genetics theory that is well tested and verified for markers with such inheritance.

Management Tasks

The tools described above provide valuable means for characterizing and classifying populations, families, and individuals to facilitate management choices. Insight gained in this program provides guidance for development of future goals and objectives.

Advice to Coleman National Fish Hatchery

Given the primary premise that the propagation program at Coleman National Fish Hatchery is to spawn the greatest number of wild fish (within the limit of 15% of the population or 20 fish whichever is greater), but control the relative contribution of gametes to the next generation so as to equalize contribution from each parent, specific advice to Coleman was to:

- Keep milt and eggs separate and make only single-pair crosses.
- Divide eggs from a female into two lots to be fertilized by the sperm from two males, whenever possible.
- Maintain maternal or paternal half-sib families separately for as long as possible, preferably until tagging and transfer.
- If the number of families has to be reduced (to create tank space) or if families have to be combined (to maintain optimal feeding), then remove or combine half-sib families in a manner that maximizes the number of parents and minimizes the number of parents shared by the remaining family groups.

Many of these guidelines were similar to ones in place at Coleman National Fish Hatchery since 1990 (S. Foott, USFWS, personal communication).

Genetic analysis of parents in each brood year helps to identify those maternal half-sib families that can be readily distinguished on the basis of diagnostic paternal alleles.

Parentage Analysis

Over 90% of BY91 offspring selected for spawning were successfully allocated to family on the basis of DNA markers (Hedgecock *et al* 1995). BY92-BY95 captive broodstock have been PIT-tagged by family. This identification is verified using genetic markers in those fish that actually mature. With such pedigree information, gametes from captive broodstock can be used in crosses that avoid inbreeding and maximize founder-allele representation. In 1995, the first year in which captive broodstock spawned successfully at Bodega Marine Laboratory, mating protocols were developed as spawning occurred (Table 2). This task is not always straightforward, however, and it becomes increasingly complicated as the pool of candidate spawners grows. Also, a large number of alternative mating scenarios must be considered as fish actually spawn; adaptive "expert" software should be developed to help make these decisions.

Escapement of hatchery-origin winter chinook to the Sacramento River in 1995 (USFWS 1996) was noted from eight coded-wire tags recovered from carcasses recovered on Battle Creek in May through August 1995. These eight fish came from six families formed in 1992; three were from the same family (F-14) and the other five came from different families (B-1, N-7, B-3, N-7, H-5). Since there were 22 matings crossed in this year, this recovery does not appear biased to any particular family. We expect a need to use genotype and pedigree information from each brood year to allocate certain winter-run returns that may not be (need not be) coded-wire tagged to family.

Sex- and Family-Related Fitness Differences

Rates of maturation and survival in captive broodstock were monitored for differences that might be related to sex or family. At low resolution, maturation and survival rates for the 12 BY91 captive families did not appear significantly different at age-3 (Figure 6). A strong bias in the sex ratio noted at this time (about eight females to one male) and, looking back, at 2 years of age compared to the relatively equal ratio at stocking (Figure 7) alerted us to the greater complexity of the situation. By going back to tissue samples collected from early mortalities and using *Oty* and microsatellite loci to assign, respectively, sex and family and referring to the fish health group reports for gonadal maturity and cause of death information, we were able to collate enlightening observations. Briefly, we found that: males from cross

8xC (8=male, C=female) had a significantly lower rate of mortality than males in all other families (Figure 8). Females, however, had no differences in rate of mortality across all families (Figure 9). This difference in rate of mortality accounts for the female-biased sex ratio at 2 and 3 years (Figure 7).

This greater fitness of family 8xC can be noted even during early life stages, where family 8xC experienced only 8% mortality compared with an average of 46% mortality for all other families. Male 8, however, was the only "non-jack" used in the matings performed in 1991, implying that perhaps

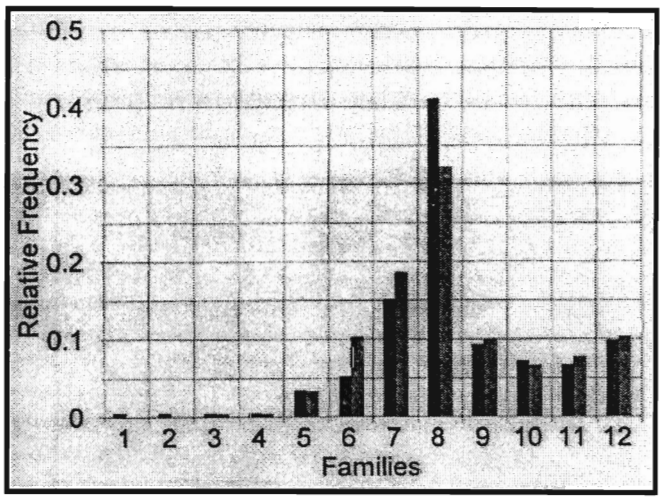

Figure 6
RELATIVE STOCKING AND SURVIVAL FREQUENCIES FOR EACH OF THE FAMILIES PROPAGATED IN BROODYEAR 91, GIVEN A SOMEWHAT RANDOM SUBSAMPLING OF SURVIVORS AT TWO YEARS
Families 1-12 are: 4xD, 4xE, 4xG, 4xH, 6xB, 6xC, 7xC, 8xC, 9xL, 9xM, 10xD, 10xJ.
Stocking frequency is shown with dark hatching.
Living at 2 years is shown with lighter hatching.
Chi square = 14.5; Probability = 0.20

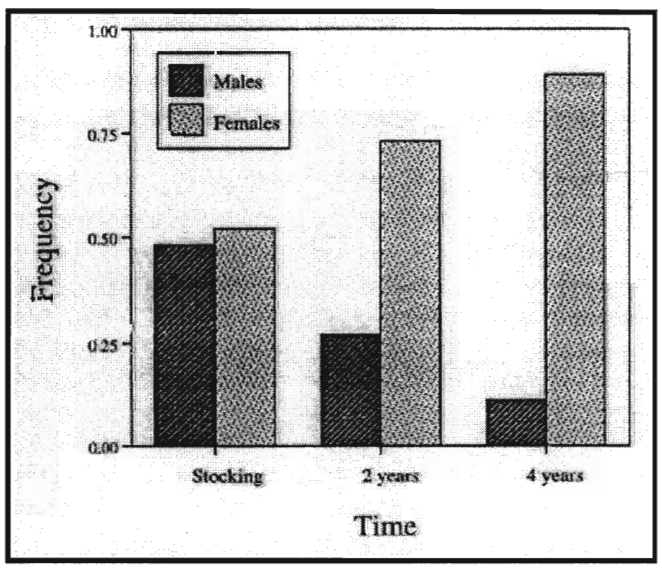

Figure 7
RELATIVE FREQUENCY OF BROODYEAR SURVIVORS, BY SEX, AT 2-YEAR INTERVALS AT BODEGA MARINE LABORATORY
The rate of male mortality exceeds that of females.

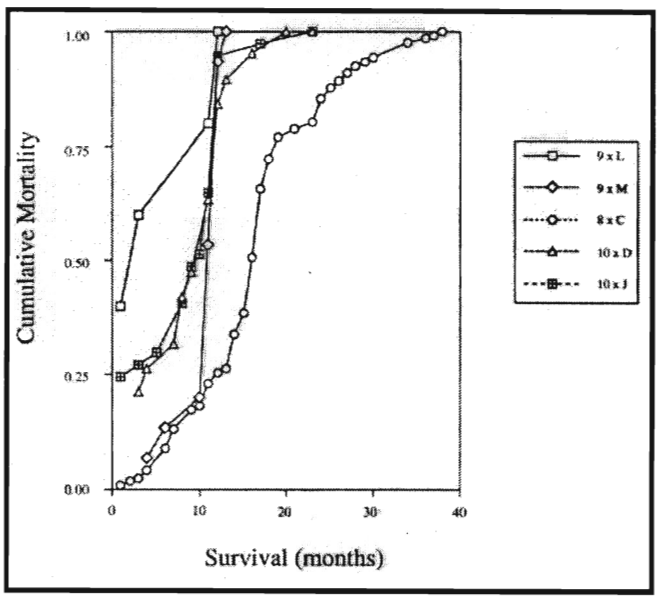

Figure 8
SURVIVAL CURVE OF BROODYEAR 91 MALES FROM DIFFERENT FAMILIES
Males from cross 8xC had significantly lower mortality rates than males from other families

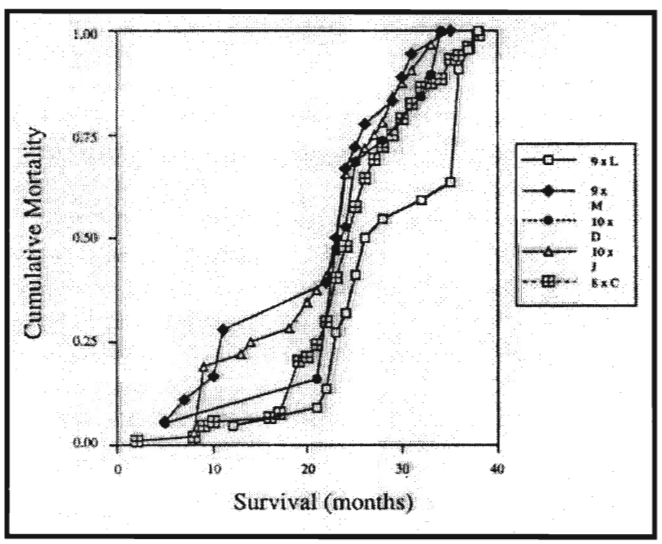

Figure 9
SURVIVAL CURVE OF FEMALES FROM ALL FAMILIES AT BODEGA MARINE LABORATORY
Females from all families had similar rates of mortality.
Typically, females lived longer than males, but unlike 8xC male offspring, females from 8xC showed a similar pattern of mortality to females of other families.

the higher rates of mortality in the other families may relate to different genetic resources spawned from jacks. The rates of mortality of families in Lot 1, which includes 6xC and 7xC (among others) has not yet been fully resolved but seem likely to hold great interest as these share the same father as family 8xC. Relative fitness among these families and in comparison with the other families may be enlightening. No significant correlation between rate of male mortality and precocious gonadal development was apparent. Data for possible correlation between rate of mortality and incidence of disease are being processed but are not yet available. A complete analysis of this information is underway and being prepared for publication.

Effective Population Sizes

An important concern of the winter-run captive breeding program is the potential impact of artificial propagation on the genetic health of the wild population. This problem has been considered in detail by Hedrick *et al* (1995), who found no evidence that the supplementation aspect of the Coleman hatchery program has reduced the overall effective population size (N_e) of the natural run. However, to monitor and document the program's genetic impacts, better estimates of the effective size of the naturally spawning winter-run population, as well as that of the hatchery-produced portion of the run, are essential. Estimates of the effective size of the natural population will be obtained from genetic analyses of adult populations over time (Pollak 1983; Waples 1989) using tissue samples from captive broodstock and in-river carcasses and genetic analyses of outmigrating juveniles biopsied at Red Bluff Diversion Dam throughout the emigration period (Bartley *et al* 1992; Pudovkin *et al* 1995).

Sex and Run Identity of Wild-Caught Candidate Spawners

Small fin-clips are taken from wild-caught winter-run spawning candidates to provide DNA for verification of sex and run identity. Sex-specific DNA sequences are amplified by PCR, using primers and conditions published previously (Devlin *et al* 1994). This identifies genotypic sex well before features of sexual dimorphism become evident (Figure 10).

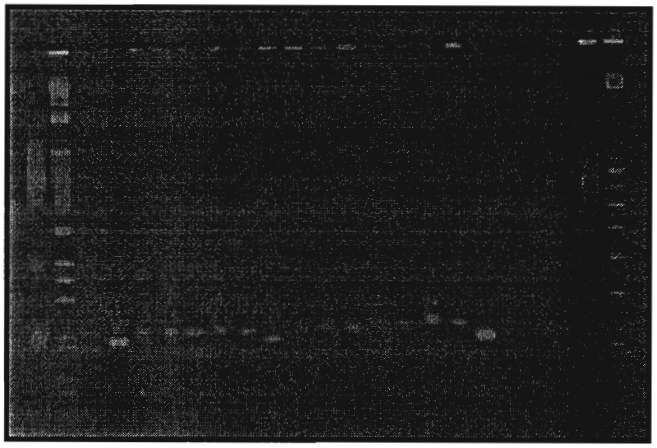

Figure 10
OTY PCR-PRODUCTS FOR SEX DETERMINATION OF BROODYEAR 91 OFFSPRING
The bright 209 base pair product is male-specific (Lanes 4, 10, 18).
All other products shown on this gel are of the female type.
A DNA size standard increasing in 100-bp increments is loaded into lane 23;
Another type of DNA size standard, loaded in lane 2, has
210 and 220 bp fragments on the same plane as the
209-bp male-specific fragment.

The genotype of each wild-caught fish is then determined for a series of polymorphic DNA markers. These genetic data were used to confirm run identity of the broodstock and parentage of any offspring they subsequently produce and will contribute, as well, to evaluation of overall genetic impacts of the captive breeding program.

A separate project, supported with funding from the Department of Water Resources, has allowed accumulation of microsatellite-DNA allele-frequency data for the four chinook spawning stocks of the upper Sacramento River (Table 4). Differentiation of winter-run from fall-, late-fall-, and spring-run populations is clearly noted from the genetic similarity tree (Figure 11)

drawn from these data using BIOSIS-1 software (Swofford and Selander 1981). Microsatellite locus *Ots-2* demonstrates this differentiation most markedly (Banks *et al* 1996). At this locus, winter-run has an "L" allele at a frequency of about 0.8, while the other runs have frequencies no greater than 0.05 (Table 4). Thus, *Ots-2* LL homozygotes, which comprise nearly two-thirds of the winter-run population, are almost certainly winter-run. On the other hand, the most common *Ots-2* genotype in the spring run, KE, is quite rare in the winter run (Tables 4 and 5; Figure 12).

In 1995, 85 adult chinook salmon were taken from the upper Sacramento River to Coleman National Fish Hatchery for artificial propagation of the endangered winter-run stock. Gametes were obtained from 41 spawned fish, but another 38 never matured and were recorded as spring-run chinook by hatchery biologists. The other six neither spawned nor were recorded as spring-run by hatchery biologists. Analyses of a microsatellite DNA polymorphism in the spawned and unspawned broodstock and in samples of fall-, late-fall-, winter-, and spring-run chinook salmon from the same drainage confirm that the unspawned fish were indeed spring run (Table 6). However, genotypic proportions in the spawned group depart significantly from random-mating expectations and in a manner suggesting admixture and, thus, hybridization of winter- and spring-run chinook. Neither the microsatellite marker nor dates of capture or first spawning allows individuals to be assigned unambiguously to run. Take of adults for artificial propagation of winter-run was, therefore, suspended until additional genetic markers can be found for run-diagnosis. Focus in our current work is on characterization of alternative microsatellite markers that may complement Ots-2's power for discrimination between the runs and increasing the number of fish in our baseline data of all populations that may be present in the upper Sacramento River when candidate winter-run spawners are captured. A thorough analysis of our findings with regard to possible spring-run chinook identified among captive spawnings in 1993, 1994, and 1995 is underway and will be published.

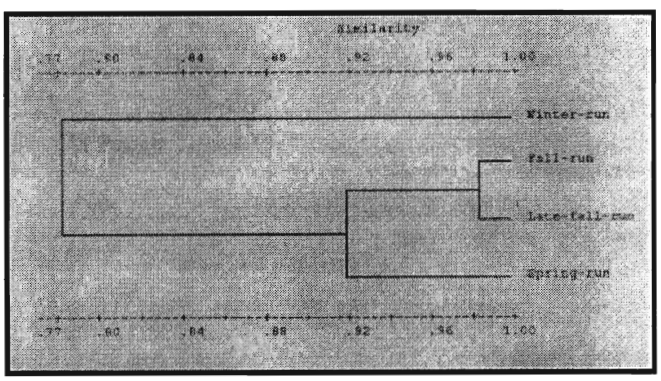

Figure 11
NEI'S GENETIC SIMILARITY OF UPPER SACRAMENTO RIVER DRAINAGE CHINOOK SALMON, AVERAGED OVER FIVE MICROSATELLITE MARKERS
From data in Table 6.
On this scale, a similarity of 1.00 would represent genetically identical populations.

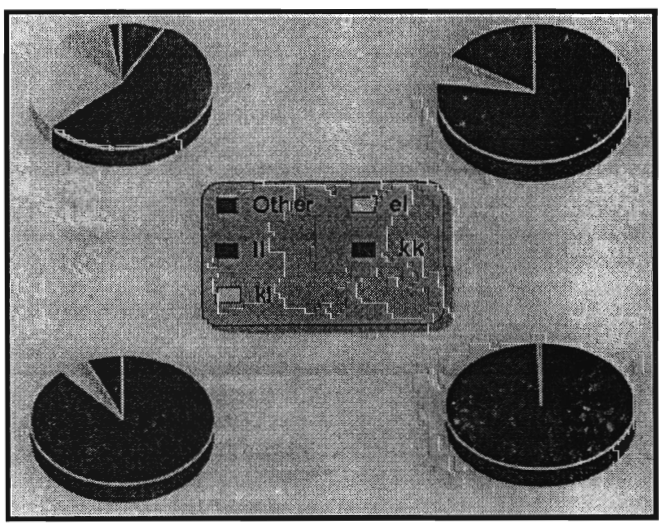

Figure 12
RELATIVE FREQUENCIES OF THE FOUR MOST COMMON WINTER-RUN GENOTYPES AT THE OTS-2 MICROSATELLITE LOCUS, SHOWING COMPARATIVE FREQUENCIES OF GENOTYPES IN EACH OF THE RUNS
Only the four most common genotypes for winter run are shown individually; all other geotypes are grouped.

Table 4
ALLELE FREQUENCY DATA FOR SACRAMENTO RIVER CHINOOK SALMON RUNS AT FIVE LOCI

Locus	Allele	Winter 91 & 94	Fall 93	Late-Fall 93	Spring 94
Ots1	Number*	67	50	44	38
	A	.649	.650	.648	.842
	B	.000	.020	.000	.000
	C	.343	.290	.330	.132
	D	.007	.040	.023	.026
Ots2	Number*	56	33	43	39
	A	.000	.106	.081	.013
	B	.009	.000	.070	.000
	C	.000	.061	.000	.013
	D	.000	.030	.023	.000
	E	.071	.303	.395	.385
	F	.000	.045	.058	.000
	G	.000	.000	.012	.064
	H	.009	.076	.000	.013
	I	.000	.015	.000	.000
	J	.009	.061	.035	.038
	K	.161	.258	.291	.410
	L	.741	.045	.000	.038
	M	.000	.000	.035	.026
Ots4	Number*	38	15	18	28
	A	.316	.000	.000	.321
	B	.184	.000	.083	.036
	C	.211	.000	.056	.107
	D	.066	.033	.083	.214
	E	.000	.000	.000	.054
	F	.013	.033	.222	.107
	G	.079	.100	.167	.161
	H	.053	.233	.111	.000
	I	.066	.167	.083	.000
	J	.000	.167	.056	.000
	K	.000	.233	.111	.000
	L	.013	.000	.000	.000
	M	.000	.033	.028	.000
Ots5	Number*	42	48	45	41
	A	.262	.031	.033	.098
	B	.738	.969	.956	.902
	C	.000	.000	.011	.000
Ots6	Number*	41	33	15	41
	A	.134	.288	.333	.146
	B	.122	.091	.000	.061
	C	.378	.288	.433	.488
	D	.366	.333	.233	.207
	E	.000	.000	.000	.037
	F	.000	.000	.000	.037
	G	.000	.000	.000	.024

*Number of individuals sampled from each run.

Table 5
ALLELIC FREQUENCIES FOR OTS-2

	Coleman Hatchery Winter-Run Stocks			Sacramento River
Allele	1995 Unspawned Winter-Run	1995 Spawned Winter-Run	Pooled 1991-1994 Winter-Run	1995 Winter-Run Carcass Samples
(N)	37	41	89	88
A	0.0	0.0	0.0	0.0
B	0.0	0.0	0.06	0.0
C	0.0	0.0	0.0	0.0
D	0.0	0.0	0.0	0.0
E	0.338	0.037	0.073	0.017
F	0.054	0.012	0.006	0.011
G	0.027	0.012	0.017	0.0
H	0.041	0.024	0.006	0.006
I	0.0	0.0	0.0	0.006
J	0.014	0.0	0.006	0.011
K	0.419	0.171	0.163	0.131
L	0.054	0.732	0.725	0.807
M	0.027	0.0	0.0	0.0
N	0.027	0.012	0.0	0.0
O	0.0	0.0	0.0	0.006
P	0.0	0.0	0.0	0.006

Table 6
NUMBERS OF OTS-2 GENOTYPES OBSERVED AND EXPECTED UNDER RANDOM MATING IN SPRING-RUN CHINOOK

Probabilities for agreement of observed and expected numbers (P{HWE}) are given in the last line.
Asterisks denote five individuals in the spawned broodstock with genotypes observed only in the Deer Creek spring-run or unspawned broodstock samples.

Geno-type	Deer Creek Spring-Run		Coleman National Fish Hatchery 1995 Broodstock				Sacramento River 1995 Winter-Run	
			Unspawned		Spawned			
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
AJ	1	0.04						
CK	1	0.39						
EE	4	6.01	5	4.11				
EF	1	1.94	1	1.47				
EG			1	0.68				
EH	1	0.39						
EJ	1	1.16						
EK	19	12.01	10	10.62	1*	0.72		
EL	1	1.55	1	1.37	3	2.92	3	2.42
EM			2	0.68				
FH			1	0.16				
FJ	1	0.19						
FK	1	1.94	2	1.70	1*	0.18		
FL	2	0.25					2	1.61
GN			1	0.05	1*	0.01		
HK			2	1.27	2*	0.36		
HL							1	0.81
IL							1	0.81
JK			1	0.42			1	0.26
JL							1	1.61
KK	4	6.01	6	6.37	2	1.35	4	1.50
KL			3	1.70	8	11.68	14	18.56
KM	2	1.16						
KN			1	0.85				
LL					27	23.37	59	57.28
LM	1	0.15						
LO							1	0.81
LP							1	0.81
Total	40		37		45		88	
P{HWE}		0.064		0.451		0.002		0.542

Chapter 4

FUTURE DIRECTIONS

Research objectives of the Winter-Run Chinook Captive Breeding Program for the immediate future are dictated to a large extent by the nature of recent experiences and challenges detailed above. Future directions for each of the main segments of the program are summarized in this chapter.

Coleman National Fish Hatchery

The Fish and Wildlife Service has completed a review of options for overcoming the imprinting of winter chinook reared at Coleman National Fish Hatchery on Battle Creek rather than the mainstem Sacramento River (USFWS 1996b). In 1996, the decision will be announced as to which strategy will be adopted, so that planning and construction of any new facilities can begin immediately and be completed in time for the 1997 spawning.

Captive Broodstock Program

The primary challenge now faced by the captive broodstock program is how to elicit normal sexual maturation of captive broodstock (that is, maturation in synchrony with that of wild-caught broodstock) and production of gametes capable of normal fertilization and development. Two complementary approaches will be made to this challenge in 1996.

First, the diet of captive broodstock will be modified from natural sources (krill and anchovy). Older year classes (BY91-BY94) that have been kept on this diet still refuse to take pelletized diets recommended (by Dr. Ron Hardy, National Marine Fisheries Service, Seattle) to provide proper balance of protein and lipids as well as vitamins. Such pelletized diets will be used on future brood years, but an alternative strategy is required for the older year classes. Preliminary experiments, in which the natural diet was coated with a mixture of cod-liver oil and vitamins, have indicated that the older fish will accept a modified natural diet. Such experiments will continue in 1996 and 1997.

A second approach is to use hormone implants to accelerate and synchronize gonadal maturation. Implants of $LHRH_a$ (from AquaPharms, Inc.) will be administered on an experimental basis with the help of Dr. Penny Swanson, National Marine Fisheries Service, Seattle. Gonadal responses of fish implanted with hormones will be followed by ultrasound observation, and the quality of any gametes ultimately spawned will be ascertained.

Finally, the poor fertilization success of cryopreserved milt indicates more research is needed in this area. Cryopreservation of milt:

- Would allow gametes to be saved from the many males that mature and succumb early in the captive rearing system.
- Would facilitate movement of gametes from the captive rearing program to Coleman National Fish Hatchery.

- Would facilitate matings among year classes for maximization of effective population size.
- Might allow reductions in captive broodstock inventory at times.

Other problems that must be addressed include: poor egg (or female) quality; low egg production from captive females; high male mortality; and low egg fertilization rates.

A final objective in the near term will be to bring ozonation of sea water on line to reduce the risk of disease transmission within the facility.

Pathology

Preliminary experiments with erythromycin injections in 1995-96 suggest that BKD may be brought under control. These experiments will continue in 1997, when spawning of wild-caught fish will resume. One improvement in the regulatory climate has been relaxation by the Food and Drug Administration of the need to obtain INADS for such treatment of protected species.

Future research on the rosette agent includes development of a vaccine, development of a DNA probe for early detection and tissue localization, and cooperation with Arizona State University on a study of association between susceptibility to rosette agent infection and genotype at certain loci of the major histocompatibility complex.

Genetics

The primary objective of the genetics laboratory in 1996 will be development of a high-power molecular diagnostic test of winter-run identity. Such a test will be needed in 1997 to avoid admixture of different runs in collection of winter-run broodstock from the Sacramento River and, thus, the potential for artificial hybridization of different runs. This is being accomplished by completing development and population surveys of new microsatellite markers in chinook stocks of the upper Sacramento River drainage. Several markers are required, like the Ots-2 marker, that show marked allele-frequency differences among runs. Multiple diagnostic markers will reduce to very low levels the chance of misclassifying other chinook salmon as winter run.

The genetics laboratory will continue to verify through microsatellite markers the parentage of all fish used in spawnings and crosses. Also, effects and interaction of family and sex on survival will continue to be monitored to evaluate the extent to which selection during the rearing of captive broodstock might modify the genetic composition of the winter-run. Typing of individuals for major histocompatibility genes will also be used to evaluate differential susceptibility to stress and disease in artificial rearing.

Finally, the genetics laboratory will obtain multi-locus microsatellite data on young winter chinook captured at Red Bluff Diversion Dam to estimate the effective size of the natural population. This parameter is needed to evaluate the impact of captive breeding on the effective size and genetic diversity of the natural population (Hedrick *et al* 1995).

Education and Outreach

The program will contribute directly and indirectly to a public education effort by the California Academy of Sciences and Bodega Marine Laboratory, funded by the National Science Foundation, to use conservation of Pacific salmon as a model for teaching about biodiversity and its preservation.

The program will continue to provide internships for local high school students who have interest in salmon conservation.

Chapter 5

PROGRAM IMPLEMENTATION

The Winter-Run Chinook Salmon Captive Broodstock Program is a multi-agency attempt to help keep winter chinook salmon from extinction, and funding reflects this participation. In 1993, the U.S. Congress passed HR 2457, authorizing the winter-run captive broodstock program at a level of $1 million annually. Initial funding was from state and federal fishery agencies.

The Department of Water Resources has provided critical logistical support by administering the combined funding from a variety of agencies in single annual contracts. This arrangement reduces the complexity of multiple contracts requiring separate administrative tasks and start dates. It also takes advantage of an existing overhead arrangement between the University of California and Department of Water Resources under which indirect costs are contained to 10%. In this manner, the Department of Water Resources, Bureau of Reclamation and (beginning in 1996) Category III funds (Urban Water Contractors administered through Metropolitan Water District) were also commingled for economy and efficiency. This is an important consideration for a project that has multiple tasks that need to maintain discrete budgets.

Similarly, in 1993 the Department of Water Resources collaborated with the University of California and National Fish and Wildlife Foundation to meet requirements of a $130,000 award that necessitated matching funds. The Department of Water Resources arranged to contract with the National Fish and Wildlife Foundation to commingle its funding that year to satisfy the matching-fund requirement. Without this assistance from Department of Water Resources, much of the work would have been curtailed due to the higher overhead costs.

The project operates on a lean budget. Each year, new and unpredictable responses to fish health or genetic requirements put added strain on a budget that averages 0.80 of budgeted costs. Much of the shortfall translates into the contracted facilities (Bodega Marine Laboratory and Steinhart Aquarium) absorbing added labor expenses. Additionally, rebudgeting occurs continually, making calendar-year planning and long-term facility modification and improvements difficult.

Five-Year Assessment

In addition to this project summary and progress report, a coastwide workshop on the status of captive breeding was held at Bodega Marine Laboratory in March 1996. The Technical Committee suggested an outside review to assess the accomplishments and future direction of the project. Bodega Marine Laboratory personnel offered, as an alternative, a 2-day workshop to include current and planned captive breeding programs in an agenda reviewing each project in a standard format. Five keynote speakers were invited to discuss topics in various fields that define current salmon captive breeding challenges.

The workshop had several objectives:

- To foster better communication between existing and planned projects.
- A thorough assessment of the potential of captive breeding as a restoration tool.
- Publication of a paper describing the workshop's consensus on the biological implication of salmonid captive breeding that would serve as a benchmark in the professional fisheries literature summarizing the promise and limitation of captive breeding.

The workshop hosted nine speakers, who summarized captive breeding projects in Alaska, Idaho, Washington, and Oregon. Kristen Arkush presented the Sacramento River winter-run chinook summary. In addition, a representative from AquaPharms, a private firm that develops $LHRH_a$ implants, presented information on current products and the centralization of INAD permits. A consensus of recurring problems in captive breeding include:

- Increasing fertilization rates and, in general, developing a clearer picture of reproductive physiology.
- Understanding the nutritional components of gamete quality.
- Ensuring that wild attributes are represented in the progeny.
- Ensuring that captive artifacts such as disease, imprinting problems, age to maturation, environmental influences on life history characteristics, and differential sex mortality can be minimized.
- Ensuring that the genetic resource represented by the wild population is not compromised by captive breeding.

The last session was a facilitated dialogue leading to a list of consensus statements that will formulate the basis of the paper summarizing the workshop. One point of concurrence was that any risk of a captive breeding artifact is acceptable if the alternative is extinction.

Publications

Banks, M.A., B.A. Baldwin, and D. Hedgecock. 1994. Progress in discrimination among California's Central Valley chinook salmon stocks using microsatellite DNA. *Proceedings of the 1994 Northeast Pacific Chinook and Coho Salmon workshop. Salmon Ecosystem Restoration: Myth and Reality.* (Eds. M. Keefe and P. Lawson.) November 7-10, Eugene, Oregon.

Banks, M.A., B.A. Baldwin, and D. Hedgecock. 1996. Research on chinook salmon stock structure using microsatellite DNA. *Bull. Natl. Res. Inst. Aquacult.*, Suppl 2:5-9.

Hedgecock, D., M.A. Banks, B.A. Baldwin, D.J. McGoldrick, and S.M. Blankenship. Pedigree analysis of captive broodstock for an endangered chinook salmon, using simple tandem-repeat DNA polymorphisms. Accepted pending minor revision, *Conservation Biology.*

Hedrick, P.W., D. Hedgecock, and S. Hamelberg. 1995. Effective population size in winter-run chinook salmon. *Conserv. Biol.* 9:615-624.

Published Abstracts

These are three published abstracts resulting from AFS meetings, one at the national meeting, two at the Western Section. I think they should be included in the publication section under a separate abstract heading. WRCCB personnel were invited to talk at each of the meetings.

Arkush, K.D and P.A. Siri. 1995.

~~Speaker~~, Conservation Hatcheries/Threatened and Endangered Species Propagation Symposium, 125th Annual American Fisheries Society Meeting, August 27-31, 1995, Tampa, Florida, USA. *The Sacramento River winter run chinook captive breeding project: molecular markers to public education.* ~~K.D. Arkush (presenter) and P.A.~~ Siri.

Dave White, D. 1995

American Fisheries Society Western Section

~~Speaker~~, ~~Cal/Neva~~ ~~Amer. Fish~~. Soc. ~~Annual~~ Meeting, Napa, CA, February 4, 1995. *An Overview of the Sacramento Winter Run Chinook Captive Broodstock Program: A Multi-Agency Effort.* ~~D. White~~

Siri, P. 1995.

~~Speaker~~, American Fisheries Society Western Section Meeting, Napa, California. "Qualitative issues in the conservation biology of Pacific salmonids: Education at all levels." February 1995. P. Siri

Education Projects

During the formative stages of the Winter-Run Chinook Salmon Captive Broodstock Program, the committee created an educational subcommittee to address societal issues relating to degradation of salmon habitat. There was strong consensus within the committee that elaborate and complex genetic salvage projects would be meaningless if the habitat problems were not given adequate attention.

The subcommittee began discussions with Ideas In Motion, a San Francisco-based firm that produced a successful Public Broadcasting Station NOVA program in 1993. Ideas in Motion began working with Bodega Marine Laboratory and California Academy of Sciences investigators to explore possibilities. This collaboration led to a series of National Science Foundation preproposals to the Informal Science Education Program, which resulted in an award of $516,900 in 1995. The focus of the project is to develop a series of static exhibits and interactive video that illustrate the problems facing society in preservation of aquatic biodiversity. The exhibits will focus on salmon, with specific interpretation of technologies being used in the Winter-Run Chinook Salmon Captive Broodstock Program. Three exhibits would be produced. One would be at the California Academy of Sciences for a few years and one would travel the country and possibly Canada for 5 years. A smaller version would be housed at Bodega Marine Laboratory.

The National Science Foundation award requires matching funds and cost sharing. A significant amount of cost sharing is represented in efforts by California Academy of Sciences and Bodega Marine Laboratory participants, but additional funding is being sought from private foundations. In discussions with private foundations a new education agenda began to materialize. Although most of the private foundations were enthusiastic about the National Science Foundation-funded project, there was more interest in developing a curriculum that would include those who influence habitat decisions. In this way, the private foundations could support the required matching funds, but most of their funding would go toward a summer institute that would target resource managers, legislative staff, and educators who would benefit from short courses focusing on conservation biology and the technologies and theory of molecular genetics.

In March 1996, Bodega Marine Laboratory hosted a meeting of 26 people representative of the target groups and researchers interested in developing the summer institutes. A preliminary organizational schematic was adopted that outlined the relationships between the National Science Foundation's informal science education project and the more technical summer institutes. Bodega Marine Laboratory hosts two meetings annually of the Bodega Field Conference, a 5-year series of technical workshops in field ecology. The logistics and format of the Bodega Field Conference is a perfect model for the summer institutes. The principal investigator, Professor Barbara Bentley of the State University of New York, Stonybrook, also conducts a field course for decision-makers and has agreed to serve as a co-investigator for the summer institutes.

Additionally, there has been a strong collaborative educational tie to the Sacramento River Discovery Center in Red Bluff. The Discovery Center is a line item of the CVIP and seeks to educate school groups and the public about preservation of Sacramento River resources and how to manage conflicts between fisheries and agriculture. The Discovery Center has requested the educational committee assist its curriculum by having the summer institute provide training to teachers in biodiversity issues. The Discovery Center will also receive one of three models to be produced by the National Science Foundation project.

Related Projects and Collaboration

A multi-year assessment of visible implant tags was initiated in 1993 using non-winter-run chinook in an effort to determine if a substitute tag could be used that would allow unique identification at a size less than the 55mm nominal threshold used for PIT tagging.

A number of project personnel have become involved in the experimental analysis of the biological effects of mass marking techniques. A pilot project was initiated in collaboration with Lee Blankenship of the Washington Department of Fisheries and Wildlife and Lee Weber of the University of Nevada-Reno to determine if heat shock proteins could be used to measure sub-clinical levels of stress resulting from different tagging techniques. After successful initial trials, a formal proposal was submitted to support multiple-year trials on chinook in various stages of smoltification and condition factors. The goal of this work is to provide a logical basis for the selection of mass marking techniques appropriate to development of a selective fishery.

Microsatellite PCR-primer sequences for Ots-1 through Ots-6 have been made available to the following:

Dr. Linda Park
National Marine Fisheries Service
Seattle, WA

Brian Neff and Dr. Mart Gross
University of Toronto, Canada

Drs. Kristi Miller and John Nelson
Dept. of Fisheries and Oceans
Pacific Biological Station
Nanaimo, BC

Ales Snoj
University of Ljubljana
Slovinia

Dr. Paul Bentzen
University of Washington

Dr. John Wright
Marine Gene Probe Laboratory
Nova Scotia, Canada

Dr. Anne Kapuscinski
University of Minnesota
St. Paul, MN

Dr. Nevin Aspinwall
St. Louis University
St. Louis, MO

Tim Kim and Dr. Phil Hedrick
Arizona State University

Dr. Roy Danzmann
University of Guelph
Canada

Dr. Kim Scribner
National Biological Survey
Anchorage, AK

Dr. Jane Symmonds
Southern Ocean Seafood Ltd.
New Zealand

Drs. Einar Nielsen and Michael Hansen
Denmark

Clone sequences for the microsatellites isolated at Bodega Marine Laboratory were made available to Paul Bentzen and the University of Washington.

We have enjoyed using microsatellite PCR-primer sequences developed in following laboratories:

Marine Gene Probe Laboratory
Nova Scotia, Canada
(Drs. John Wright and Paul Bentzen)

Wetlands & Estuarine Ecology
National Biological Survey
Anchorage, AK
(Dr. Kim Scribner)

Department of Zoology
University of Guelph
Guelph, Ontario, Canada
(Dr. Roy G. Danzmann)

Vera, Please add sections provided by Paul

Thanks -
I haven't gotten any other comments. Ready for press.

27 Nov. '96

Randy:

I think it is important that we include the information below not only because it describes collaborations and project evolution, it also illustrates how the WRCCB project operates within the scientific community making published contributions on several levels. Meetings like this are, in themselves, important milestones and WRCCB funding made them happen. I would suggest these paragraphs be inserted on page 37 in the section on related projects and collaboration.

I include the introductory comments to the aquaculture colloquium which acknowledges DWR support. I believe you have a full set of the papers published in Conservation Biology along with our introductory comments. If you don't, let me know and I'll have a set sent to you.

I also suggest we show the three attached abstracts resulting from invited AFS talks. We were solicited for the national meeting as well as the western section and demonstrates, I believe, a growing recognition of the project within the community of professional fisheries biologists. They could be included under publications (pg. 34) under a separate heading of: Published Abstracts. There are others but these are more noteworthy.

Have a good holiday,

Paul Siri

Two colloquia focussing on topics of concern in captive breeding were held at the Bodega Marine Laboratory in 1993 and 1994 supported by funding from the National Fish and Wildlife Foundation and the Department of Water Resources as part of the winter run project. The 1993 colloquium invited fourteen speakers to explore the relationship between life history variation and quantitative genetics. The meeting was held September 8-12, 1993 and titled "Conservation Biology of Endangered Pacific Salmonids: Life History, Genetics and Demography" and was published as a special section of *Conservation Biology, 8 (1994) 863-864.*

Endocrinology

The following year BML held as its annual colloquium a meeting titled "Applications of ~~Pacific~~ Rim ~~Aquaculture~~ to Pacific Rim Aquaculture" which contained a special evening workshop directed at the problems associated with maturation and fertilization in salmonid captive breeding programs. This was published as an abstract: *Siri, Paul and Johnson, K., Maturation and Reproduction in salmonid Captive Breeding Programs, Aquaculture, 135 (1995) 217-218.* Both these meetings shaped the direction of the captive breeding program.

The 1993 colloquium contributed greatly to the models of winter run effective population size and the 1994 colloquium identified the weaknesses in reproductive biology in salmonid captive breeding. The collaborations that developed as a result of these meetings also created the underpinnings of the 1996 coast wide workshop on salmonid captive breeding.

Pedigreed DNA from the families spawned in 1991 have been made available to:

Dr. Kim Scribner
National Biological Survey
Anchorage, AK
(Transmission genetics for isoloci; paper in preparation.)

Tim Kim
Arizona State University
(Characterization of MHC loci with a strong potential for development of markers for run discrimination.)

Ales Snoj from the Biotechnical faculty, University of Ljubljana, Slovinia visited our laboratory for about a month to study biotechnology techniques in cloning and PCR. Recent news indicates that, following his return home, he was the first scientist to develop microsatellite loci for marble trout.

A senior undergraduate student, Ken Queener, made a preliminary study of the ocean catch at Bodega Bay in 1995. He screened fish sampled from fishermen for the LL Ots-2 genotype characteristic of winter chinook salmon. No LL individuals (likely winter run) were discovered in the 200 fish sampled.

High school students who have visited and worked in the molecular genetics laboratory to experience the flavor of careers in science are:

Zach Burt
Rancho Cotati High School

Oona Squire
Summerfield Waldorf School

Demonstration and assistance with photonic tagging took place at the United Anglers' hatchery at Casa Grande High School in Petaluma, California.

Other high school students who worked with the broodstock group are: Janeen Gold, Mike Gold, Jamie O'Hara, Jim Haberson, Alex Iezza, Ben Jones, Ben Perry, Karina Salonger, Kim Tingstrom.

REFERENCES

Amos, B., C. Schloterer, and D. Tautz. 1993. Social structure of pilot whales revealed by analytical DNA profiling. *Science* 260:670-672.

Banks, M.A., B.A. Baldwin, and D. Hedgecock. 1994. Progress in discriminating among California's Central Valley chinook salmon stocks using microsatellite DNA. In: *Salmon Ecosystem Restoration: Myth or Reality?* Proc. of the 1994 NE Pacific Chinook and Coho Salmon Workshop. Salmon Ecosystem Restoration: Myth and Reality. M. Keefe and P. Lawson, ed., Am. Fish. Soc. special publ.

Banks, M.A., B.A. Baldwin, and D. Hedgecock. 1996. Research on chinook salmon stock structure using microsatellite DNA. chinook salmon stocks using microsatellite DNA. *Bull. Natl. Res. Inst. Aquacult.* Sppl 2:5-9.

Bartley, D., M. Bagley, G. Gall, and B. Bentley. 1992. Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. *Conserv. Biol.* 6:365-375.

Bingham, N., and S. Barnes. 1992. *Preliminary Report on Winter-run Chinook Salmon Captive Breeding Program.* (U.S. congressional update by the Winter-Run Chinook Captive Broodstock Committee). 25 pages.

Bullock, G.L., H.M. Stuckey, and D. Mulcahy. 1978. Corynebacterial kidney disease: egg transmission following iodophore disinfection. *Fish Health News* 7:51-52.

Cobb, B.D., and J.M. Clarkson 1994. A simple procedure for optimising the polymerase chain reaction (PCR) using modified Taguchi methods. *Nucleic Acids Research* 22(18):3801-3805.

Devlin, R.H., B. Kelly-McNeil, I.I. Solar, and E.M. Donaldson. 1994. A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of chinook salmon. *Aquaculture* 128:211-220.

Donaldson, E.M., G.A. Hunter, and H.M. Dye. 1981. Induced ovulation in coho salmon (*Oncorhynchus kisutch*). II. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. *Aquaculture* 26: 129-142.

Donaldson, E.M., G.A.Hunter, H.M. Dye, and G. VanDerKraak. 1985. Induced ovulation in Pacific salmon using LH-RH analogs and salmon gonadotropin. *Current Trends in Comparative Endocrinology* (eds B. Lofts and W.N. Holmes). Hong Kong, Hong Kong University Press. Pp.375-377.

Edwards, A., A. Civitello, H.A. Hammond, and C.T. Caskey. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am. J. Hum. Genet.* 49:746-756.

Elliott, D.G., and D.F. Amend. 1978. Efficacy of certain disinfectants against infectious pancreatic necrosis virus.

Elston, R.A., L. Harrell, and M.T. Wilkinson. 1986. Isolation and *in vitro* characteristics of chinook salmon (*Oncorhynchus tshawytscha*) rosette agent. *Aquaculture* 56:1-21.

Evelyn, T.P.T., L. Prosperi-Porta, and J.E. Ketcheson. 1986. Experimental intra-ovum infection of salmonid eggs with *Renibacterium salmoninarum* and vertical transmission of the pathogen with such eggs despite their treatment with erythromycin. *Diseases of Aquatic Organisms* 1:197-202.

Fisher, F.W. 1994. Past and present status of Central Valley chinook salmon. *Conservation Biology* 8(3):870-873.

Fryer, J.L., and J.E. Sanders. 1981. Bacterial kidney disease of salmonid fish. *Annual Reviews in Microbiology* 35:273-298.

Greenberg, A.E., L.S. Clesceri, and A.D. Eaton, editors. 1992. *Standard Methods for the Examination of Water and Wastewater.* 18th edition. Pages 9-38 - 9-39. Victor Graphics, Inc., Baltimore, Maryland, USA.

Harrell, L.W., R.A. Elston, T.M. Scott, and M.T. Wilkinson. 1986. Significant new systemic disease of net-pen reared chinook salmon (*Oncorhynchus tshawytscha*) brood stock. *Aquaculture* 55:249-262.

Healy, M.C. 1991. Life history of chinook salmon *(Onchorynchus tshawytscha).* Pages 313-393 in *Pacific Salmon Life Histories.* C. Grout and L. Margolis, editors. University of British Columbia Press.

Healy, M.C. 1994. Variation in the life history characterization of chinook salmon and its relevance to conservation of the Sacramento winter run of chinook salmon. *Conservation Biology* 8(3):876-877.

Hearne, C.M., S. Ghosh, and J.A. Todd. 1992. Microsatellites for linkage analysis of genetic traits. *Trends Genet.* 8:288-294.

Hedgecock, D., M.A. Banks, B.A. Baldwin, D.J. McGoldrick, and S.M. Blankenship. 1995. Parentage analysis of captive brood stock for an endangered chinook salmon, using simple tandem-repeat DNA polymorphisms. *Conserv. Biol.*, accepted pending revision.

Hedrick, P.W., D. Hedgecock, and S. Hamelberg. 1995. Effective population size in winter-run chinook salmon. *Conserv. Biol.* 9:615-624.

Hedrick, R.P., C.S. Friedman, and J. Modin. 1989. Systemic infection in Atlantic salmon *Salmo salar* with a *Dermocystidium*-like species. *Diseases of Aquatic Organisms* 7:171-177.

Hughes, C.R., and D.C. Queller. 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Mol. Ecol.* 2:131-137.

Kerk, D., A. Gee, M. Standish, P.O. Wainwright, A.S. Drum, R.A. Elston, and M.L. Sogin. 1995. The rosette agent of chinook salmon (*Oncorhynchus tshawytscha*) is closely related to choanoflagellates, as determined by the phylogenetic analyses of its small ribosomal subunit RNA. *Marine Biology* 122:187-192.

Lichatowich J., L. Mobrand, L. Lestelle, and T. Vogel. 1995. An approach to the diagnosis and treatment of depleted Pacific salmon populations in Pacific Northwest watersheds. *Fisheries* 20:10-18.

Mitchum, D.L., and L.E. Sherman. 1981. Transmission of bacterial kidney disease from wild to stocked hatchery trout. *Canadian Journal of Fisheries and Aquatic Sciences* 38:547-551.

Moyle, P.B. 1993. Saving California's salmon: The legacy of Ishi. *Trout: Journal of Coldwater Fisheries Conservation* 34(3):14-17.

Moyle, P.B. 1994. The decline of anadromous fishes in California. *Conservation Biology* 8(3):869-870.

Nehlsen, W. 1994. Salmon Stocks at risk: Beyond 214. *Conservation Biology* 8(3):867-869.

Nehlsen, W., J.E. Williams, and J.A. Lichatowich. 1991. Pacific salmon at the crossroads: Stocks at risk from California, Oregon, Idaho, and Washington. *Fisheries* 16(2):4-21.

National Marine Fisheries Service. 1990. Endangered and threatened species; Sacramento River winter-run chinook salmon. *Federal Register* 46515 Vol 55(214). November 5, 1990.

Pollak, E. 1983. A new method for estimating the effective population size from allele frequency changes. *Genetics* 104:531-548.

Pudovkin, A.I., D.V. Zaykin, and D. Hedgecock. 1995. A new, indirect estimate of the effective number of breeders based on genotypic data for a single cohort. *Genetics*, in review.

Queller, D. C., J.E. Strassmann, and C.R. Hughes. 1993. Microsatellites and kinship. *Trends Ecol. Evol.* 8:285-288 + centerpage.

Reimers, E., P. Landmark, T. Sørsdal, E. Bøhmer, and T. Solum. 1986. Determination of salmonids' sex, maturation and size: an ultrasound and photocell approach. *Aquaculture Magazine* 13: 41-44.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press.

Swofford, D.L., and R.B. Selander. 1981. Biosys-1. A computer program for the analysis of allelic variation in genetics. Dept of Genetics and Development. Univ of Illinois at Urbana-Champaign, Urbana, Illinois.

Tautz, D. 1990. Genomic fingerprinting goes simple. *BioEssays* 12:44-46.

Tautz, D., and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nuc. Acids Res.* 12:4127-4138.

Thoesen, J.C., Editor. 1994. *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens.* 4th ed., Version 1, Fish Health Section, American Fisheries Society.

U.S. Fish and Wildlife Service. 1989. An Operational Plan to Utilize Coleman National Fish Hatchery to Propagate Winter-Run Chinook Salmon. Red Bluff, CA. 9 pages.

U.S. Fish and Wildlife Service. 1992. Coleman National Fish Hatchery Winter Chinook Salmon Broodyear 1991 Production Report.

U.S. Fish & Wildlife Service. 1993. *Biological Assessment of the Effects of Coleman National Fish Hatchery Operations on Winter-Run Chinook Salmon.* Northern Central Valley Fish and Wildlife Office. Red Bluff, CA. 56 pages.

U.S. Fish and Wildlife Service. 1995. *Fish and Wildlife Service Manual.* Part 713, Fish Health — Fish Health Policy.

U.S. Fish and Wildlife Service. 1996. *Escapement of Hatchery-Origin Winter Chinook Salmon (Oncorhynchus tshawytscha) to the Sacramento River, California in 1995, with Notes on Spring Chinook Salmon in Battle Creek.* Northern Central Valley Fish and Wildlife Office. Red Bluff, CA.

U.S. Fish and Wildlife Service. 1996b. *A Review of Rearing and Release Strategies to Improve Imprinting, Homing and Survival of Hatchery Origin Sacramento River Winter Chinook-Salmon, Oncorhynchus tshawytscha.* North Central Valley Fish and Wildlife Office, Red Bluff.

U.S. Fish and Wildlife Service, U.S. National Marine Fisheries Service, U.S. Bureau of Reclamation, and California Department of Fish and Game. 1988. Agreement to implement actions to benefit winter-run chinook salmon in the Sacramento River. 12 pages.

Waples, R.S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121:379-391.

Weissenbach, J., G. Gyapay, D. Dib, and A. Vignal. 1992. A 2nd-generation linkage map of the human genome. *Nature* 359:794-801.

Weil, C., R. Billard, B. Breton, and B. Jalabert. 1978. Pituitary response to LH-RH at different stages of gametogenesis in the rainbow trout (*Salmo gairdneri*). *Annales de Biologie Animale Biochimie Biophysique* 18:863-869.

Williams, J.E., and C.D. Williams. 1991. The Sacramento River winter chinook salmon: Threatened with extinction. Pages 105-115 in *California's Salmon and Steelhead: The Struggle to Restore an Imperiled Resource.* A. Lufkin, editor. University of California Press.

Wolf, K., and C.E. Dunbar. 1959. Test of 34 therapeutic agents for control of kidney disease in trout. *Transactions of the American Fisheries Society* 88:117-134.

UNIVERSITY OF CALIFORNIA, DAVIS

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

(707) 875-2211
FAX: (707) 875-2089
INTERNET: UCDBML@UCDAVIS.EDU

BODEGA MARINE LABORATORY
P.O. BOX 247
BODEGA BAY, CALIFORNIA 94923

31 October 1996

Vera Tharp
9317 Spring Valley Rd
Marysville
CA 95901

Hello Vera

Herewith the figures for the technical report. A few details.

Figure 6 - 9 & 11 are simple, I enclose two copies as they should be in the final with no changes.

Figure 10 has a photograph.
I enclose two copies of the photograph. It would be preferable to have the darker one in the report as it does not have any writing on it. The lighter one may indeed be the one you decide to go with as I imagine it would reproduce better. In either case, could you ask the photographic folks to avoid the ruler on the left hand side and the bottom. The photograph in the report should zero in on the actual gel. PLEASE could you send the photographs back to us when done as they are part of our lab notes. Thanks.

Figure 12
I enclose a copy of figure 12 but do not want you to use this one. I have asked Sheila Greene to send you one with better shading. She kindly offered her skills in graphics and given that I'm no good at this, I took her up on it. The one I enclose is from a slide presentation in color an thus not much good in B&W.

That's it!

Please call me if you need anything else (707 875 2077). Thanks for your help.

Sincerely
Michael
Michael